VOLUME 18 NUMBER 2

NUTRITION REVIEWS

February 1960

Poultry Nutrition Research, by C. R. Grau 33

Clinical Nutrition

Clay- and Cornstarch-Eating Women 35
Hypoglycemia Induced by Galactose 38
Vanadium Inhibition of Cholesterol Synthesis in Man 39
Uric Acid Degradation in Man 42
Vitamin B_{12} and Growth of Children 45
Phenylacetic Acid and the Production of Serotonin 47

Animal Nutrition

Volatile Fatty Acids for Correction of Low Fat Milk 48

Experimental Nutrition

Dairy Products and Dental Caries 49
Dietary Fat and Hepatic Lipogenesis 51
An Enzymatic Function of Carnitine 52
Molybdenum Toxicity 54
Dietary Fatty Acid Interrelationships 57
Methionine Metabolism 58
Riboflavin Requirements of the Cat 60
Utilization of Hydroxyanthranilate 61

Notes

Letter to the Editor 63
Vitamin Interrelationships 63
Decreased Density of Bone: An Etiologic Approach to Diagnosis ... 64
Botulism 64
Recent Books 64

Published Monthly at Mt. Royal and Guilford Aves., Baltimore, Md.

for

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

COPYRIGHT, 1960 NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, One Shattuck Street, Boston, Mass.

Assistant Editor

ELIZABETH CHAMBERLIN, One Shattuck Street, Boston.

Associate Editors

R. W. ENGEL, PH.D., Department of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg.
ALFRED E. HARPER, PH.D., Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.
ROBERT E. HODGES, M.D., Department of Internal Medicine, State University of Iowa, Iowa City.
STANLEY LEVEY, PH.D., Departments of Biochemistry and Surgery, College of Medicine, Western Reserve University, Cleveland.
JAMES F. MEAD, PH.D., Department of Physiological Chemistry, School of Medicine, University of California, Los Angeles.
OLAF MICKELSEN, PH.D., Laboratory of Biochemistry and Nutrition, National Institute of Arthritis and Metabolic Diseases, Bethesda.
WILLIAM J. PYLES, M.D., New York Heart Association, Inc., New York.
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston.
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester.
DONALD M. WATKIN, M.D., National Institutes of Health, Bethesda.
ROBERT W. WISSLER, PH.D., M.D., Department of Pathology, University of Chicago.
CALVIN W. WOODRUFF, M.D., Department of Pediatrics, Vanderbilt University Medical School, Nashville.

Editorial Committee

WALLACE R. AYKROYD, M.D., Food and Agriculture Organization
GEORGE R. COWGILL, PH.D., Yale University
CHARLES S. DAVIDSON, M.D., Boston City Hospital
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
IAGO GALDSTON, M.D., New York Academy of Medicine
WENDELL H. GRIFFITH, PH.D., University of California
CHARLES GLEN KING, PH.D., Nutrition Foundation
BERWYN F. MATTISON, M.D., American Public Health Assn.
L. A. MAYNARD, PH.D., Cornell University
HAZEL STIEBELING, PH.D., Institute of Home Economics
LEROY VORIS, PH.D., National Research Council
WARREN WEAVER, PH.D., Rockefeller Foundation
JOHN B. YOUMANS, M.D., Office of the Surgeon General, Washington, D. C.

Subscriptions should be sent to The Nutrition Foundation, Inc., 99 Park Ave., New York 16, N. Y.
Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Second-class postage paid at Baltimore, Md.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the origional meaning of the review is preserved.

NUTRITION REVIEWS

VOL. 18 FEBRUARY 1960 No. 2

POULTRY NUTRITION RESEARCH

In recent years, a remarkably short time has elapsed between the discovery of new information and its application in applied poultry nutrition. This reversal of the usual state of affairs, in which scientists have had to convince non-scientists of the merit of their findings, has found feed manufacturers who read trade magazines using new facts several months before they have appeared in scientific journals.

This ever-present pressure of fact application has had mixed results. In its favor has been its success in achieving efficient production of poultry meat and eggs. In opposition to it, however, is its diversion of many eager minds from imaginative paths of investigation to more pedestrian ones. This has been particularly evident in recent years, when, for example, the headlong search for unidentified growth factors has involved many more investigators than would seem to be justified, even on the practical grounds of improving the quality of feeds.

As the interest in applied nutrition has surged from one popular topic to another, many worthwhile studies have been virtually abandoned. This has been particularly noticeable when natural food sources of a nutrient can be supplemented economically by a manufactured product. Some examples of this are: (1) The importance of manganese in poultry diets was discovered over 20 years ago but for the last 15 years almost no research has been done to uncover its nutritional significance; it is no longer a practical problem. (2) The mode of action of antibiotics in stimulating growth is perhaps a less clear-cut example, but there has been a noticeable decrease in interest in determining how antibiotics work, now that their practical usefulness has been evaluated. (3) Several vitamins and minerals have not received enough attention because the practical problem of diet sources has been solved.

This introduction to the present status of poultry nutrition may seem to be an unduly critical one, but it is certainly not meant to be. In the past, poultry nutritionists have often led those working with other animals into new areas of discovery. We need to continue maintenance of a balance between research that may have future usefulness (fundamental research) and that which may be used immediately (applied or practical research).

Chemically defined diets. One measure of success in nutrition is the effectiveness of purified or completely defined diets in replacing foods of natural origin. Poultry nutritionists have gone far toward reaching this goal, and sufficient information is now available to permit good growth and reproduction of birds fed purified diets. Diets in which all components are of known composition (defined or synthetic diets) are not yet completely satisfactory replacements for diets composed of natural materials, but it is doubtful that even defined diets lack any major components. As better defined diets become available, the quality of nutritional research can be improved and the special roles of natural or processed foods can be more easily studied.

Amino acids. An excellent example of nutritional advances following development of highly purified diets can be seen respecting the amino acids, the need for which continues to interest many investigators. After the first task of assessing the qualitative requirements, interest has shifted to the interrelationships between amino acids and other nutrients, and among the amino acids themselves. Problems peculiar to

birds (especially the essentiality of arginine in the diet) are only part of the story. Because of the cost and limited availabilities of pure amino acids, most workers have used natural or processed proteins as the principal source of amino acids and have supplemented these with appropriate amino acids.

This approach has given, as an important by-product, information on the nutritional values of various protein sources, particularly such concentrates as the oil seed meals, fish meals and meat meals. As the amino acid deficiencies of these foods were uncovered, the possible use of synthetic or isolated amino acids has been of interest. Methionine has been available in quantity for several years, but its evaluation as a feed supplement is difficult when levels are marginal, and its costs have been high enough not to encourage widespread use in replacement of protein sources of methionine.

It is apparently not appreciated by many workers that estimation of the dietary requirement for a nutrient does not have the same biological significance as an LD_{50}, or the level of amino acid needed to prevent change in body weight. The quantitative requirement must be based on using some arbitrary criterion for deciding at what level the requirement is met. This becomes very important when decisions have to be made about supplementation of natural diets with amino acids.

Poultry nutritionists are searching for methods of evaluating protein sources in terms of the amounts of available amino acids they contain. The need here is a very practical one for a rapid chemical or bioassay method that will yield the amounts of each individual amino acid present in forms available to the animal. The problem is not limited to amino acids, of course, but these are particularly important in applying nutritional information to diet formulation.

Minerals. The sort of complex problems that are ahead of nutritionists are exemplified by recent research on the minerals zinc and selenium. The dietary need for each of these is influenced very much by other components of the diet. Levels required in diets cannot be expressed without reference to the other constituents. There is a low but significant dietary requirement for zinc that, in chicks, is fairly easily satisfied by natural foods. When some purified proteins are included in the diet, however, indigestible bonds are formed with zinc, and several times the usual level of zinc must be added if an effective zinc level is to be maintained. Turkey poults are even more sensitive to this than are chicks.

Selenium, by contrast, has not been shown to be an essential nutrient, yet supplements of it are needed with certain diets containing torula yeast. Selenium plays a role in the very involved need for antioxidants such as vitamin E.

Other minerals besides zinc and selenium have been subjected to renewed scrutiny, primarily concerning their interrelationships for normal growth and egg production. Little attention has been paid to dissolved minerals (water quality) as compared to minerals supplied as feed mixtures.

Meeting energy needs. As the scientific approach to poultry feeding developed in the early part of the century, information from other farm animals was applied to chickens. The needs for energy were considered to be most important, and various attempts were made to evaluate feedstuffs. These were only moderately successful, however, because of intervening needs for vitamins and amino acids; while these were being studied, some work was being done on the energy metabolism of chickens and the productive or net energy of feedstuffs. The time was not right for extensive interest, however, until the late 40's, when growth and efficiency of feed utilization became important to both geneticist and nutritionist.

It was some time before the importance of dietary energy levels to protein and other nutrients was realized. Some workers felt

that good growth could be obtained only with rations high in energy; indeed, the idea that bulky feeds were valueless for poultry was widely held. Then came a series of investigations in which the diets were diluted markedly with indigestible materials such as oat hulls or wood cellulose. These studies showed that despite great dilution, birds were able to consume enough feed to maintain almost normal growth, but the body fat content was reduced. It is now generally believed that the energy level of the feed is the primary determinant of food intake, and that this in turn, greatly affects the utilization of dietary protein and other nutrients.

Attempts have been made to formalize the relationship between energy and protein as the so-called "calorie-protein ratio". In this ratio, the number of calories of productive energy per pound of diet are divided by the per cent of crude protein in the diet. The faults of this ratio have been pointed out by a number of workers, several of whom have commented on its hybrid nature and inadequacy in application. The simplest criterion to apply to figures that purport to express useful relationship is their *prediction value*. The "calorie-protein ratio" fails this test.

That there is some significant relationship between the utilizable energy and other constituents of the diet is unquestioned, especially as they are related to voluntary food intake. At present, however, an adequate measure of these interrelations has not been expressed in any simple terms.

In birds, digestion trials are complicated by simultaneous voiding of urine and feces. This anatomical consideration and the observed variation in productive energy values of feedstuffs has led to the conclusion that, for birds, metabolizable energy is a more useful estimate of usable feed energy than is productive energy. Although much has been written concerning energy utilization by poultry, very little new information on the energy values of feedstuffs has been obtained. Only one or two laboratories in the United States have undertaken the task of providing accurate figures.

Species studied. Poultry nutritionists now use several birds with widely varying needs as useful tools in research. Chickens are most popular, but turkeys, ducks, geese, pheasants, quail, and pigeons have also been studied. There is increasing interest in hereditary differences in nutrient needs. A new experimental bird that shows great promise in all types of poultry research is the Japanese button quail, *Coturnix japonica*, which attains sexual maturity in less than two months and which lays eggs at a high rate. These eggs are much heavier in proportion to the hen's body weight (a 10 g. egg from a 100 g. hen) than those of chickens (a 56 g. egg from a 2000 g. hen).

One of the oldest experimental materials for biological research is the chick embryo, which still continues to be a very useful animal. Some research techniques for directly studying the nutrition of the embryo show promise of increasing the variety of nutritional problems that may be studied with chick embryos.

C. R. Grau, Ph.D.
Department of Poultry Husbandry
University of California, Davis

CLAY- AND CORNSTARCH-EATING WOMEN

Clay- and cornstarch-eating pregnant and nonpregnant women from rural Alabama reveal the socio-economic, cultural and dietary factors associated with their bizarre practices.

Pica, a craving for unnatural articles of food, and geophagy, the practice of eating earthy substances (especially clay), have attracted the attention of curious observers

for centuries. Many hypotheses on the origin of pica and of geophagy in particular have been proposed. Some suggest that they are intuitive efforts on the part of the practitioners to compensate for nutritional deficiencies, but most regard them as cultural phenomena founded in superstition and perpetuated by tradition, especially in rural areas. There is ample evidence that pica is more prevalent among pregnant women. However, women are more influenced by cultural and social factors during pregnancy than during their nonpregnant life. It is not possible, therefore, to accept unequivocally the concept that pica is a manifestation of nutritional deficiencies resulting from parasitism or the excessive metabolic demands of pregnancy.

The ingestion of clay and cornstarch is not uncommon among women in rural areas of Alabama. Since no studies of the biochemical effects of the ingestion of clay and cornstarch have been reported, C. H. Edwards *et al.* (*J. Am. Dietet. Assn.* **35,** *810 (1959)*) have initiated a series of studies designed to examine closely several aspects of this problem. The original article in this series describes the socio-economic backgrounds of the subjects and gives much information about their practices and the nutritive values of the usual diets of the clay- and cornstarch-eaters and the control subjects.

Subsequent reports will describe the relationships of clay- and cornstarch-ingestion to the results of analyses of blood and excreta of a selected sample of these women, the condition of their infants at birth and the complications during pregnancy.

The authors studied 86 women selected from prenatal and postpartum clinics. The patients were divided into six groups: pregnant and nonpregnant clay-eaters, pregnant and nonpregnant cornstarch-eaters, and, as controls, pregnant and nonpregnant women who ate neither clay nor cornstarch. Forty-two were clay-eaters, 23 were cornstarch-eaters and six ate both. Twenty-one women served as controls. A tabulation of socio-economic data revealed that the average age was 28 years (15 to 45), 74 were married, 12 were single, they had an average of 3.8 children (0 to 11), 68 were homemakers, 13 were farmers, 5 were in other occupations, the average weekly income was $39.00, the average weekly food budget was $13.00, and the eighth grade was the average level for formal education (second grade to senior year in college).

Interviews revealed similarities in practices, in motivations and in emotional responses among the clay-eaters and the cornstarch-eaters. Clay was usually obtained from nearby rivers or stream banks, although it could be purchased in some areas at the roadside. Cornstarch was usually purchased. The preferred clay was light gray, although a less smooth clay, stained pink presumably by iron salts, was used by some. One brand of laundry cornstarch selling at 8 cents per box was preferred, although one woman consumed blue liquid starch. The women paid nothing for clay but spent an average of about 15 cents weekly on starch.

The clay was usually eaten uncooked, but some was baked prior to eating, especially if it was still moist. The cornstarch apparently was consumed uncooked, although specific information on this point is lacking. Clay was eaten at various times of day, sometimes with, sometimes before and sometimes after meals. Cornstarch was eaten prior to meals or, in one instance, in place of a meal. The amount of clay consumed daily ranged from 6 to 130 g. The amount of cornstarch consumed ranged from 15 g. to 2 pounds daily.

The women gave a variety of reasons for eating clay. Some had a "taste" or a "craving" for it, while others found it to have a pleasant odor or to create a pleasant sensation in the mouth. Thirty-six clay eaters felt "satisfied" after eating clay, five

claimed it relieved the nausea of pregnancy and three maintained it prevented vomiting during pregnancy. Still others affirmed its usefulness in relieving a wide variety of physical and emotional complaints.

The women gave similar reasons for eating cornstarch. "Taste", "craving", a pleasant sensation in the mouth and a pleasant odor (especially when ironing) were common explanations. Feelings of satisfaction, release of tensions and drowsiness were the desirable subjective sensations reported to follow the ingestion of starch. Morning sickness, nausea and vomiting in pregnancy were relieved in some by eating starch.

When deprived of access to their favorite, whether clay or cornstarch, both groups testified to the presence of emotional states characterized by such adjectives as "crazy", "awful", "terrible", "troubled", "disgusted", "irritable" and "worried".

No apparent physical symptoms directly associated with clay- or cornstarch-eating were reported from this study. Clay-eating had no influence on the appetite for food of 35 women, abolished the appetite for food of five and was the *sine qua non* of appetite in at least one. Surprisingly enough, cornstarch-eating had no effect on the appetite of 17 women and actually stimulated a desire for food in one woman.

Almost all the women had "cravings" or "tastes" for food only during pregnancy, rejecting the items after the births of their children.

The women studied reported knowledge of only two men who ate clay and one man who ate cornstarch, lending some credence to the theory that these forms of pica may be sex-linked habits.

A majority of the women interviewed maintained that their clay- or cornstarch-eating was of their own choice, not the result of imposed tradition and superstition. None the less, the strong forces at work in their communities to encourage pica undoubtedly contributed at a subconscious level. Fear of harming the unborn child if clay or cornstarch were not ingested was a prime motivation among several women.

The nutritive values for the usual diets of 77 women in the study were calculated and rated according to the Recommended Dietary Allowances of the Food and Nutrition Board (*Publication 589, National Academy of Sciences-National Research Council, Washington, 1958*) as "excellent", "good", "fair" or "poor" and grouped according to the six categories of subjects. In all groups caloric intakes were below the recommended allowances and only the nonpregnant controls came close to an adequate caloric intake. In general, the diets of the control groups supplied more calories, protein, iron, thiamin, riboflavin, niacin and ascorbic acid than did the experimental groups. However all diets were low in calcium, and the diets of the pregnant clay- and cornstarch-eaters contained substantially less than recommended amounts of iron, thiamin and niacin. Fifty-four per cent of the diets of clay-eaters and 33 per cent of the diets of cornstarch-eaters were classified as "poor", whereas only 14 per cent of those of the controls were so rated. The authors suggest that the self-selected diets of the clay- and corstarch-eaters may have precipitated a need for additional nutrients which may have been supplied by clay (iron and calcium) and cornstarch (calories).

Although the interpretation of these findings as indicating nutrient deficiencies may be premature, the subsequent articles dealing with medical and biochemical data from these patients should be of interest. Additional well-controlled studies of the influence of dietary supplementation on the prevalence of pica would lend considerable support to the theory of a nutritional etiology for this bizarre habit.

The authors have, in addition to presenting their own findings, reviewed a fascinat-

ing literature on the general subject of pica. The work of M. Cooper (*Pica: A Survey of the Historical Literature as Well as Reports from the Fields of Veterinary Medicine and Anthropology, the Present Study of Pica in Young Children, and a Discussion of Its Pediatric and Psychological Implications. Charles C. Thomas, 1957*) contains historical details of pica from ancient to modern times.

HYPOGLYCEMIA INDUCED BY GALACTOSE

Pronounced elevation in blood galactose may be associated with hypoglycemia.

Galactosemia, an hereditary disorder which appears in infants, is evidenced by an increased galactose level in the blood (as shown by increased total reducing sugar levels) and by poor growth. The metabolic effects of this hereditary disorder have been reviewed earlier (*Nutrition Reviews* **14,** *5 (1956)*). Because of an accumulation of galactose-1-phosphate, it is believed that galactosemia is due to a deficiency of a specific enzyme (galactose-1-phosphate uridyl transferase) which allows further metabolism of this compound (*Ibid.* **17,** *115 (1959)*).

Recently, T. L. Dormandy, D. Leak and M. Grant (*Lancet* **II,** *269 (1959)*) have studied the effect of galactose on the level of glucose in the blood. Using the glucose oxidase method for the determination of blood sugar, it was possible for these investigators to determine changes in the glucose content of the blood when there were large quantities of galactose or other reducing sugars present.

In studying six volunteers, each of whom was given an oral galactose tolerance test after taking 50 g. of galactose, transient slight falls in blood glucose were noted in only three of the six individuals. After receiving 100 g. of galactose by mouth, four of the six volunteers showed only a slight decrease in blood glucose during the first hour following the ingestion of the sugar. However, when a high blood galactose level was obtained by giving the galactose intravenously, five of the six volunteers showed depressions in the blood glucose levels within a few minutes after the galactose had reached its peak value (total reducing sugar about 325 mg. per cent). Under these conditions the maximal decrease in the blood glucose amounted to approximately 30 mg. per cent and was persistent for about 90 minutes.

Thus it appears that with this group of volunteers (apparently adults) the elevation of the total blood sugar produced by giving galactose intravenously caused a fall in the blood glucose levels.

Since lactose (hydrolyzed to galactose) may be less efficiently utilized in the neonatal period than it is in the adult, these investigators carried out preliminary studies on six prematures and three full-term infants. They did not believe that galactose tolerance tests, either oral or intravenous, need be carried out on these newborn babies for this series of studies. However, they studied the galactose and glucose levels while the infants were maintained on their ordinary feeding schedules, and in this manner employed a "rough and ready" galactose and glucose tolerance test. In the case of seven of the nine infants, there was an increase in the blood glucose levels in the hour and a half following the feedings.

However, in one premature infant and one premature and jaundiced infant there were marked decreases in the blood glucose along with the elevation of the total blood sugar during the early period following the oral feeding. On the second day of life for the first infant, the fasting blood sugar was 47 mg. per cent and it fell to a minimum value of 15 mg. per cent 45 minutes after the feeding. By the third day of life the

tolerance of galactose had increased so that, in spite of a minimum blood glucose value of 19 mg. per cent reached 15 minutes after feeding, the blood glucose returned to the fasting level within one hour after feeding, remaining at approximately the same level for the next hour.

The tests were repeated on the tenth and twentieth days and, in both cases, the blood glucose increased greatly after feeding with only small increases in the total reducing sugar levels.

Similar trends were noted in the second premature infant who was jaundiced, and in this case, while the fasting blood glucose was 32 mg. per cent on the third day of life, it fell to a minimum value of 13 mg. per cent 30 minutes after feeding and attained a fasting value about 30 minutes later. During this time, the total blood sugar (representing mainly galactose) steadily increased, reaching a maximum value at 30 minutes (90 mg. per cent). On the fourth day of life, similar levels of blood glucose were noted, but by the ninth day there was an increase in the blood glucose level after ingesting food and the galactose was at its maximum, only 20 mg. per cent.

While none of these individuals showed definite clinical signs of hypoglycemia, the possibility could not be excluded that hypoglycemia produced by galactose could be a clinical as well as a biochemical feature in the neonatal period. The chemical detection of such a hypoglycemia would require the use of the glucose oxidase method.

The means by which the increased blood galactose influences the blood glucose is not known. The possibility exists that the blood galactose stimulates the pancreas directly to produce increased amounts of insulin. Also, the release of glucose by the liver may be influenced by the elevated blood galactose. These investigators suggest that the metabolic regulatory system responsible for maintenance of the blood glucose within its normal narrow limits apparently cannot readily differentiate between glucose and galactose. Thus a sharp rise in the blood galactose will cause a decrease in the blood glucose level.

VANADIUM INHIBITION OF CHOLESTEROL SYNTHESIS IN MAN

Vanadium inhibition of cholesterol synthesis in man resulted in lowering of serum cholesterol. Balance studies indicated that tissue cholesterol stores were also reduced.

Treatment of atherosclerosis has been primarily concerned with reduction of serum cholesterol. This has followed the demonstration that a significant correlation exists between coronary atherosclerosis and elevated serum cholesterol (D. Davis, B. Stern and G. Lesnick, *Ann. Int. Med.* **11,** *354 (1937)*).

There are at least three ways by which body cholesterol may be lowered. First, the degradation of cholesterol to bile acids may be increased, but there is apparently no non-toxic way of doing this at present. Also, primary absorption of dietary cholesterol or reabsorption of biliary cholesterol can be diminished. The result is a readjustment of synthesis by the liver to compensate for the decrease in cholesterol returned to the circulation. Thus it appears that decreasing the synthesis of cholesterol is the best method for controlling the cholesterol content of the body.

One method of reducing synthesis is by decreasing the intake of fat. Also, the ingestion of increased amounts of unsaturated fatty acids will reduce synthesis. In view of the difficulty encountered in trying to change food habits, the use of an inhibitor which can interfere with some step in the intermediary synthesis of cholesterol may prove to be advantageous. G. L. Curran and D. L. Azarnoff (*Arch. Int. Med.* **101,**

685 (1958)) have discussed the properties of an ideal inhibitor. According to their reasoning, inhibition should precede the step at which squalene cyclizes to form the steroid nucleus. Sterols other than cholesterol have been found in atherosclerotic plaques. On the other hand, blocking the synthesis of acetyl-coenzyme A from acetate would have little influence on cholesterol synthesis since acetoacetate, which follows in the sequence, is also derived from fat catabolism.

Salts of vanadium have the unique property of inhibiting the utilization of mevalonic acid for cholesterol synthesis according to Azarnoff and Curran (*J. Am. Chem. Soc.* **79,** *2968 (1957)*). Curran and R. L. Costello observed that deposition of cholesterol in rabbit aorta was inhibited by vanadium salts (*J. Exp. Med.* **103,** *49 (1956)*). In a recent review (*Nutrition Reviews* **17,** *231 (1959)*) it was disclosed that workers subjected to vanadium fumes or dust show a significantly lower serum cholesterol when compared with fellow workers not so exposed.

Curran, Azarnoff and R. E. Bolinger (*J. Clin. Invest.* **38,** *1251 (1959)*) have recently examined the effects of vanadium on sterol balance in normal men on a rigidly controlled diet.

The subjects were five normal male medical students, ages 23 to 26. All were fed identical portions of a diet which was constant throughout the study. After a two-week control period, 100 or 125 mg. of diammonium oxy-tartratovanadate were given daily for a six-week experimental period. Finally, there was a three-week recovery period without vanadium. Serum cholesterol, phospholipid, triglyceride, alkaline phosphatase and transaminase were determined. Urine analysis for vanadium, 17-ketosteroids, and 17,21-dihydroxy-20-ketosteroids was done at the beginning and end of the vanadium administration period. Three-day stool collections were frozen, lyophilized, ground and stored under vacuum prior to analysis.

During the administration of vanadium the level of serum free and total cholesterol fell significantly, while during the subsequent recovery period it rose once more. Vanadium did not appear to influence serum phospholipids, and the cholesterol to phospholipid phosphorus ratio was significantly lowered by the eighth week but was again elevated by the end of the recovery period.

Serum triglycerides rose significantly during vanadium administration and then fell after treatment was discontinued. Fecal fat did not change, indicating that vanadium did not interfere with absorption of fat from the gastrointestinal tract. Total fecal sterol excretion decreased during vanadium administration but increased again during the period without vanadium.

Vanadium balance studies show that about 60 per cent of ingested vanadium is excreted in urine in a 24-hour period, according to studies made by N. A. Talvitie and W. D. Wagner (*Arch. Indust. Hyg.* **9,** *414 (1954)*). Determinations of urinary vanadium in the report of Curran, Azarnoff and Bolinger revealed that vanadium absorption was variable from individual to individual and from time to time. At the end of vanadium administration analyses of urinary vanadium were continued along with determinations of serum free cholesterol. The per cent lowering of serum free cholesterol remained at about 20 per cent while urinary vanadium excretion fell from 80 to 20 micrograms per day. Subsequently, the lowering of serum cholesterol decreased to 13 and then to 8 per cent as vanadium excretion dropped to 15 and then 10 micrograms.

If the vanadium excretion is calculated as micrograms per pound of body weight, then a maximal lowering of free cholesterol in the serum appears to be maintained while vanadium excretion is as low as 1 microgram per pound of body weight per day.

There was no obvious evidence of toxicity from vanadium, and no changes were observed in complete blood counts, routine urinalyses, blood urea nitrogen and blood glucose during vanadium administration. Evaluation of liver function by analysis of cholesterol esters, serum alkaline phosphatase, serum transaminase and serum bilirubin showed no change. Analysis of urinary 17-ketosteroid and 17,21-dihydroxy-20-ketosteroid excretion showed no inhibition by vanadium.

The 20 per cent lowering of serum cholesterol was the maximum effect produced. However, Curran, Azarnoff and Bolinger point out that the subjects had normal cholesterol levels, and that the rate of cholesterol synthesis and the lipoprotein transport system were probably normal. In order to determine whether higher levels of serum cholesterol might be further reduced by this treatment, a clinical trial of vanadium in hypercholesterolemic individuals would be necessary.

Although this study presents no direct evidence that endogenous cholesterol synthesis has been inhibited, findings in experimental animals have indicated that this is likely. In addition, *in vitro* studies by Azarnoff, Curran and W. P. Williamson (*J. Nat. Cancer Inst.* **21,** *1109 (1958)*) have demonstrated that vanadium inhibits the incorporation of acetate-1-C^{14} into cholesterol in human intracranial tumors.

The reduction in fecal sterol output may be explained in one of three ways. The reduction could be due to an increase in retention of cholesterol in tissues with no change in endogenous synthesis. It also could be the result of a lowering of synthesis with no net change in tissue cholesterol content, or it may represent decreased synthesis and diminished tissue cholesterol stores as well as reduced output of fecal sterol.

The first possibility is unlikely in view of the experimental evidence that cholesterol synthesis is inhibited and tissue storage of cholesterol is decreased in rabbits after vanadium administration (J. T. Mountain, F. R. Stockell, Jr., and H. E. Stokinger, *Proc. Soc. Exp. Biol. Med.* **92,** *582 (1956)*). Analysis of livers of two patients with brain tumors who had received vanadium for four and eight months respectively, showed cholesterol levels of 225 and 250 mg. per 100 g. of liver. These values are below the average normal of 300 mg. per 100 g. for a man weighing 70 kg.

In order to determine whether inhibition of cholesterol synthesis has resulted in decreased tissue stores, Curran, Azarnoff and Bolinger determined sterol balances in their experimental subjects. Dietary sterol, fecal neutral sterol and fecal bile acids were determined. Urinary sterols were assumed to be negligible, serum sterols constant, and conversion to steroid hormones unaffected. By incubating feces with and without added vanadium it was demonstrated that the vanadium did not produce its effect by increasing bacterial destruction of sterol. The amount of dietary sterol absorbed was determined by comparing the control period with the experimental periods.

Dietary sterol following ingestion is partly absorbed and mingles with synthesized sterol to make up the total body sterol, which equals the sum of tissue and serum sterols. About 25 per cent of absorbed dietary sterol is excreted along with bile acids and endogenous neutral sterols. This total excreted sterol, plus unabsorbed dietary cholesterol, comprises the output from the intestines. The authors grant that the amount of absorption and excretion of dietary cholesterol could be more accurately determined if isotope techniques were possible for man. Nevertheless, assuming the basis for calculation to be sound, they believe that the trends are correct for body and tissue sterol changes.

In order to determine whether vanadium inhibition reduces tissue cholesterol levels, its synthesis during the recovery period

was calculated. The change in body sterol was assumed to be zero.

To calculate the synthesis during vanadium treatment, the ratio of excreted sterol during vanadium administration to excreted sterol during recovery was considered proportional to the ratio of synthesis values in the two periods. This assumption was based on the observation that synthesis was the major contributor to excreted sterol as well as on the fact that the calculation of the extent of reduction in synthesis gave values less than would be expected from previously mentioned isotope studies of synthesis depression in animals and humans. Since the period of greatest change, namely from the control period to the vanadium period, was not used in the calculation of synthesis inhibition, it may be assumed that the calculated values are conservative minimums.

In spite of minimizing factors, a reduction in body and tissue sterol content occurred during the vanadium period. One man showed a large reduction in total serum cholesterol while tissue sterol was increasing, showing the importance of calculating tissue sterol content.

Although the details of the calculations cannot be given here, it is evident that a great deal can be learned by careful balance studies. This method should be valuable in comparing the effects of several cholesterol lowering compounds now under active investigation. Synergistic effects should be sought between vanadium and compounds decreasing dietary cholesterol absorption.

URIC ACID DEGRADATION IN MAN

Extrarenal elimination of uric acid in man via the gastrointestinal tract is stressed. Human tissues do not have the enzyme systems to degrade uric acid, but this can be done by the intestinal flora.

For the complete degradation of uric acid, one requires enzyme systems consisting of uricase, allantoinase, allantoicase, and urease. Thus, in the stepwise breakdown of uric acid, the following compounds are formed in sequence: allantoin, allantoic acid, urea, glyoxylic acid, and finally carbon dioxide and water. Depending upon which of the various enzymes are missing in different species, purine metabolism may end with the formation of uric acid, allantoin, allantoic acid, urea or ammonia, and carbon dioxide.

The metabolism of uric acid in man has been the subject of discussion for many years. Earlier investigators reported values ranging from almost complete recovery to 28 per cent of the injected uric acid appearing in the urine, the usual average being about 50 per cent. After the induction of isotope techniques, it has been reported that between two thirds and three fourths of the isotope injected in man as uric acid can be recovered as urinary uric acid.

Recently L. B. Sorensen (*Metabolism* **8**, *687* (*1959*)) has reported studies involving the degradation of uric acid in man. Uric acid labeled in the 2 position with C^{14} was injected intravenously into five individuals who had been maintained on a purine free diet for five days before the study. This group consisted of two normal individuals, two gouty subjects and one patient with arteriosclerotic heart disease. The urine was collected from these patients for ten to 13 days following the administration of the tagged uric acid. At the end of this time the C^{14} content of the body uric acid had fallen to within a few per cent of the concentration at zero time.

Sorensen found that the intravenously injected tagged uric acid was not recovered quantitatively as urinary uric acid. In the two normal individuals recoveries of 64.3

and 68.9 per cent of the administered uric acid were obtained from the urine, while recoveries of only 46 and 46.3 per cent of the administered material were obtained in the urine of the two gouty subjects. As expected, the miscible uric acid pool in the gouty individuals was almost twice that of the normals, although both patients had only a short history of gout.

It appeared, therefore, that the mean daily urinary excretion of uric acid was less than the amount of uric acid being replaced per day by the body. For the two normal subjects the amount of uric acid in the urine was only 57.9 and 69.5 per cent of the calculated turnover of uric acid, while the values for the patients with gout were 47.8 and 45.8 per cent. From these calculations it appeared that a surplus of 262 or 213 mg. of uric acid was being formed over the amount excreted in the urine by the normal individuals, while the gouty patients had surpluses of about twice these values. On the other hand, the value for the patient with arteriosclerotic heart disease was within the normal range. Thus these observations indicated that there must be some pathway of excretion of uric acid other than a urinary route.

Since some C^{14} was found to be incorporated into allantoin, allantoic acid, urea and carbon dioxide, following the intravenous administration of uric acid, one has to assume that some breakdown of uric acid takes place in the human body. In the normal subjects it was found that 2.1 and 3.9 per cent of the injected uric acid C^{14} was recovered in urinary allantoin two and five days respectively after administration. However, in the case of the two gouty patients, 4.7 and 0.4 per cent of the isotope appeared in allantoin five and two days respectively after the tag was given.

While Sorensen did not quantitatively determine the allantoin in the urine, he estimated the amount of urinary allantoin resulting from the degradation of uric acid. It was calculated that an average of 24 mg. of urinary allantoin had been derived daily from uric acid in the four patients. Upon comparing this value to the reported average daily output of allantoin in the urine (10 to 100 mg.), one might assume that a considerable amount of urinary allantoin is derived from the actual breakdown of uric acid in the body.

After the administration of uric acid tagged with radioactive carbon, C^{14} could be noted in the allantoic acid and urea fractions of the urine. It was calculated that about 2.6 per cent of the uric acid C^{14} appeared in the urinary allantoic acid during the first 12 hours after the isotope had been given. In all five patients significant amounts of C^{14} were found in the urea. In the case of two normal individuals, 1.7 and 2.2 per cent of the injected uric acid C^{14} was found in the urinary urea 13 and 15 days, respectively, after the administration of the tagged uric acid. Similar values were found in two patients with arteriosclerotic heart disease (one new patient and the one described earlier), as well as in a gouty patient.

Should the C^{14} tagged uric acid be degraded to such an extent that carbon dioxide is formed in the body, then there should be some loss of activity in the expired air. The two normal persons excreted 4 and 11 per cent of the injected C^{14} through the lungs one and four days, respectively, after the administration of the tagged uric acid. Seven per cent of the dose was recovered in the air expired by a gouty patient over a 48-hour period.

Thus in these studies it was observed that administered uric acid is degraded in the human body and that the radioactivity in an initial dose of C^{14} labeled uric acid will appear in allantoin, allantoic acid, urea, and carbon dioxide. When C^{14} labeled uric acid was given to patients by intravenous administration, the renal excretion of uric acid amounted to only 50 to 70 per cent of the administered dose. Hence the question arises as to whether the material is ex-

creted in the digestive juices and whether there is degradation of uric acid in the intestinal tract.

Because of the lack of specific methods, Sorenson did not accept as reliable earlier reports suggesting that between 40 and 70 mg. of uric acid were excreted in the digestive juices per day and that 10 to 20 mg. of this may occur in the feces. Using the method of differential spectrophotometry, it was found that 3.1 (1.7 to 4.6) mg. per cent of uric acid occurred in the saliva of ten normal men. The average value in the saliva of 12 normal women was lower, 1.9 (0.9 to 2.8) mg. per cent. The daily excretion of uric acid in the saliva was estimated as between 30 and 50 mg. per day for men.

On two occasions it was found that saliva from a gouty subject contained 6.67 and 6.24 mg. per cent of uric acid, a value which is considerably above the normal limit. If this patient produced 1.5 liters of saliva per day, then he lost no less than 100 mg. of uric acid per day in the saliva, making it one of the major pathways of uric acid excretion.

Gastric juice was found to contain 0.5 to 1.0 mg. per cent of uric acid. Uric acid assays on sterile bile obtained from the fistulae of ten patients, in whom a choledochotomy was performed in connection with a cholecystectomy, yielded an average uric acid concentration of 4.4 mg. per cent. Thus, if a patient were producing one liter of bile per day, he would lose about 50 mg. of uric acid in this secretion.

Sorensen believes that the daily excretion of uric acid in the saliva, gastric juice and bile in normal man amounts to about 100 mg. per day, and somewhat less in women. It was postulated that if similar amounts of uric acid are expected in pancreatic and intestinal juice, then at least 200 mg. per day are lost from the body through the intestinal route. Thus, he believes that approximately one-third of the uric acid formed is disposed of through the digestive juices in normal individuals, and in the case of patients with hyperuricemia, the extrarenal elimination plays a more important role.

Sorensen also believes that the rate of uricolysis in the intestinal tract, as judged from these studies, is sufficient to account for the degradation of one third of the uric acid turned over in normal human beings.

Since uric acid is poured into the intestine by the various digestive juices, the question arose as to the role of intestinal flora in uricolysis in man. This problem was studied by determining the rate of turnover, the miscible pool size, and the recovery of C^{14} uric acid metabolic products both before and after effective bacteriostasis was induced in the intestine by the administration of antibiotics and sulfa drugs.

The miscible pools in the two normal subjects were approximately the same and the milligrams of uric acid turned over per day (699 and 710 mg.) were similar, along with urinary excretion (480 and 415 mg.) both before and after effective bacteriostasis was established. Sixty-nine per cent of the administered C^{14} was recovered as urine uric acid initially, and after the intestine was sterilized the value dropped to 55.7 per cent. During the control period 7.2 per cent of the injected dose of radioactivity was found in the feces, but since no uric acid could be demonstrated, it was assumed that the activity was due to C^{14} incorporated in the bacteria and degradation products of uric acid.

After the bacteriostasis had been well established uric acid was constantly found in the feces, and a total of 930 mg. was excreted over a five day period following the injection of the purine. This corresponds to the 26.6 per cent of the total uric acid turnover, while at the same time 30 per cent of the amount of the injected activity was recovered in the stool, indicating that essentially all the C^{14} in the feces was incorporated in uric acid.

Before antibiotics were given to the

patient who received the uric acid intravenously, 2.1, 0.2, 2.2 and 10.9 per cent of the administered dose of radioactivity was found in the urinary allantoin, allantoic acid, urea, and in the carbon dioxide expired in the air, respectively. Following the sterilization of the intestine, however, only 1.8, essentially none, 0.7 and 0.5 per cent of radioactivity was found in the corresponding urinary fractions. Before sterilization 7.1 per cent of the dose of uric acid C^{14} was found in the stool as degradation products, while after sterilization essentially none was found. Thus, with an active intestinal flora, 22.5 per cent of the uric acid C^{14} was recovered in different metabolic degradation products. This dropped to 3 per cent after a high degree of intestinal bacteriostasis was established. The author suggests that the intestinal flora has an important role in the degradation of uric acid in man.

Next, Sorensen studied whether or not there was uricolytic activity in human tissue, *i.e.*, processes which will break down uric acid to form simpler products. To study this, he used uric acid tagged in the C-6 position with radioactive carbon. Samples of liver, kidney, spleen, pancreas and whole blood (all obtained at autopsies) were tested to see whether or not, upon incubation of the homogenate of the tissues with the tagged uric acid, there would be an increased radioactivity in the carbon dioxide produced. Sixty-four different experiments were carried out with different human tissues and the amount of carbon dioxide liberated in all cases was negligible. Thus it would appear that uric acid is not broken down in the human tissues tested, possibly indicating that the enzyme systems responsible for the breakdown of uric acid do not exist in these tissues.

This work may be summarized as follows. Uric acid is not enzymatically degraded in human tissues. However, a considerable amount of uric acid is excreted into the gastrointestinal tract where it may be broken down by the bacteria present in the flora. Extrarenal excretion eliminates a considerable portion of the uric acid produced each day.

VITAMIN B_{12} AND GROWTH OF CHILDREN

Oral administration of 20 micrograms of vitamin B_{12} daily to underdeveloped children in Central America failed to alter their rate of increase in height or weight.

Since 1949, when vitamin B_{12} therapy was reported to stimulate the growth of children (N. C. Wetzel *et al.*, *Science* **110,** *65 (1949)*; *Nutrition Reviews* **8,** *139 (1950)*) there has been continuing interest in the possibility that vitamin B_{12} deficiency might be a fairly general cause of growth failure (*Nutrition Reviews* **10,** *146 (1952)*; **11,** *42 (1953)*). During the past ten years there have been many attempts to test this possibility and the published reports of these tests have been reviewed by E. E. Howe (*Am. J. Clin. Nutrition* **6,** *18 (1958)*). The results taken all together are inconclusive, since both negative and positive results were obtained.

A further study of the effect of oral administration of 20 micrograms of vitamin B_{12} daily to Guatemalan children has been reported by N. S. Scrimshaw, J. A. Muñoz, O. B. Tandon and M. A. Guzmán (*Am. J. Clin. Nutrition* **7,** *180 (1959)*). Their experiment merits attention since it was particularly well controlled and was carried out on children having an extremely low intake of animal protein, whose development was from two to four years behind that of well-nourished children of comparable age.

The experimental subjects were 278 school and preschool children in five small rural villages. They were ranked according

to age and sex and were assigned randomly to placebo or experimental groups. Their daily intakes of animal protein ranged from 8 to 14 g. They ate ad libitum in their own homes throughout the study and, six days a week, were given orally tablets containing 20 micrograms of vitamin B_{12} or a placebo tablet identical in appearance. Even the personnel administering the tablets did not know which children were receiving vitamin B_{12}.

Height and weight measurements were taken monthly for one year on a group of 50 of the preschool children, and for 18 months on four groups (228 individuals) of the school children, except for twelve weeks when they were on vacation. The height and weight gains were adjusted for initial differences in age, height and weight by an analysis of covariance.

The results obtained in the experiment with preschool children gave no indication that vitamin B_{12} administration had influenced the rate of increase in either height or weight. The results on the four groups of school children were calculated for three successive six-month periods to permit twelve comparisons. In ten of these no effect of vitamin B_{12} was discernible; in one comparison a significant positive effect was obtained and in another a significant negative effect was obtained. In one trial in the experiment on school children the average increases in height, with and without vitamin B_{12}, were 0.37 and 0.36 cm. per month, respectively, and gains in weight of 0.20 and 0.18 kg. per month, respectively.

The authors conclude from these results and from a survey of the available literature on the subject, that vitamin B_{12} supplementation is without effect on the nutritional status of children from technically underdeveloped areas whose growth and development is retarded.

They discuss some of the problems in studies of this type, emphasizing the need for extreme care in selecting the most reliable control. Differences in the growth rates of children in the different villages make it clear that almost any type of result could be obtained by using children of one village as the control group and those from another as the experimental group. Also, large differences in the growth rate of a single group of children from one six-month period to another precluded comparisons between successive periods (with and without supplementation) where each group served as its own control.

Moreover, the authors emphasize the fallacy of reporting beneficial effects of supplementation on a few individuals in an experimental group who show unexpectedly large responses, since, in their study, such individuals were found with equal frequency in both experimental and control groups. Some of these problems have been discussed previously (*Nutrition Reviews* **16,** *287 (1958)*).

The main criticism that can be made of experiments of this type on underdeveloped children is that raised by Howe (*loc. cit.*), *i.e.*, would these children have responded to the treatment if some other dietary factor had not been limiting the response? Their experiment does not answer this criticism but Scrimshaw *et al.* cite a previous study (Scrimshaw and M. A. Guzman, *Nutrition Symposium Series No. 7, p. 101 (1953)*; *Nutrition Reviews* **12,** *271 (1954)*) in which dietary supplements were included with vitamin B_{12} to make up for existing deficiencies and in which, again, no beneficial effect of vitamin B_{12} could be demonstrated.

These observations offer a challenge to those who believe that vitamin B_{12} administration may produce a growth response in well-nourished children whose growth and development is retarded for their age. The Committee on Nutrition of the American Academy of Pediatrics attempted to resolve the question by undertaking several critical and carefully controlled experiments with children, but no convincing evidence was found that vitamin B_{12} promotes growth (*Nutrition Reviews* **16,** *287 (1958)*).

PHENYLACETIC ACID AND THE PRODUCTION OF SEROTONIN

The increased production of 5-hydroxytryptamine (serotonin) could be reduced in certain individuals by feeding phenylacetic acid, believed to act as an inhibitor of 5-hydroxytryptophan decarboxylase.

The role of serotonin (5-hydroxytryptamine) in various biological reactions has been reviewed earlier (*Nutrition Reviews* **15,** *156* (*1957*); **16,** *85* (*1958*)). In certain carcinoid tumors of the large bowel, excessive amounts of serotonin are being produced which get into the general circulation and are responsible for many of the symptoms such as diarrhea and flushing which occur in these patients. It is believed that serotonin is produced biologically from 5-hydroxytryptophan. A. A. Davison and M. Sandler (*Nature* **181,** *186* (*1958*)) have shown that some of the aromatic acid metabolites of phenylalanine inhibit *in vitro* the decarboxylation of 5-hydroxytryptophan, thus decreasing the rate of formation of 5-hydroxytryptamine.

With this in mind, Sandler and H. G. Close (*Lancet* **II,** *316* (*1959*)) studied the effect of administering phenylacetic acid to a patient who had carcinoid tumors which were secreting 5-hydroxytryptophan. Five grams of phenylacetic acid were given to this patient after obtaining a one hour urine collection, and urines were then collected at periods of one hour for the next 11 hours. Under similar conditions, a patient was studied who did not receive the phenylacetic acid.

All the urine samples were analyzed to determine the amount of 5-hydroxyindoleacetic acid and 5-hydroxytryptamine excreted per hour. 5-Hydroxyindoleacetic acid was determined since it is believed to be the index of serotonin metabolism. A decrease in both the 5-hydroxytryptamine and the 5-hydroxyindoleacetic acid was noted after the patient received the phenylacetic acid, although the decrease in the indoleacetic acid was greater than that of the serotonin. This would indicate that there was an *in vivo* inhibition of 5-hydroxytryptophan decarboxylase.

As well as analyzing the effects of a single dose, these investigators gave the patient 6 g. phenylacetic acid per day for a period of five successive days. Since actual values for the urinary excretion of serotonin or 5-hydroxyindoleacetic acid are not given for this period, one must assume that there was no inhibition of the production of serotonin when more than a single dose of phenylacetic acid was given.

In the second study, M. Sandler, A. Davies and C. Rimington (*Lancet* **II,** *318* (*1959*)) observed the effect of administering 5 g. of phenylacetic acid on the urinary output of 5-hydroxyindoleacetic acid in five patients with the carcinoid syndrome during an 11-hour period following the administration of the acid. In three of the five patients there was a marked decrease in the levels of 5-hydroxyindoleacetic acid found in the urine, caused, the authors believe, by the inhibiting effect of phenylacetic acid on 5-hydroxytryptophan decarboxylase, an enzyme capable of producing serotonin from 5-hydroxytryptophan.

To study the effect of daily doses, two of the individuals with carcinoid tumors received 6 g. of phenylacetic acid per day for six successive days. However, the output of 5-hydroxyindoleacetic acid was the same as during the control period. One of these two, however, had a marked response to the inhibitor on the 11-hour single dose test while the other had no response. Also, three patients who had received phenylacetic acid in a double blind test for 14 successive days exhibited no significant difference in the flushing or diarrhea when compared with the placebo periods.

The question arose as to why the phenylacetic acid was an effective inhibitor of the

production of serotonin when studied over a short period (11 hours) but had no effect when given over a longer period. While at present there are a few suggested explanations, there is little evidence to prefer one over the other. In explaining the varied ability of phenylacetic acid to inhibit the production of serotonin in different patients, the authors suggested that perhaps there may be differences in the ability of the phenylacetic acid to penetrate the tumor mass sufficiently rapidly to allow for the accumulation of an effective inhibition level.

However, it should be noted that M. D. Milne (*Lancet* **II,** *467 (1959)*) questions whether the phenylacetic acid inhibits the 5-hydroxytryptophan decarboxylase *in vitro*, or whether there is a temporary blocking of the renal excretion of the 5-hydroxyindoleacetic acid.

At present this problem is not solved, but it is unlikely that it will be without a solution for long.

VOLATILE FATTY ACIDS FOR CORRECTION OF LOW FAT MILK

Volatile fatty acids fed to cows producing low fat milk as a result of low roughage diets at least partially restore the butterfat level of the milk.

The well-established observation that diets low in roughage and high in concentrates will usually result in the production of low butterfat milk by dairy cattle has aroused considerable interest because of the bearing this has on both normal ruminant and non-ruminant fat metabolism. The ruminant differs from the non-ruminant in the large quantities of fatty acids absorbed directly from the rumen-reticulum. These fatty acids are metabolized as a source of energy and, in dairy cattle, are converted into butterfat to a considerable extent.

The marked disturbance in fatty acid synthesis occurring in the rumen-reticulum with high-concentrate low-roughage diets has led to experiments in which dairy cattle have been fed the volatile fatty acids (sodium acetate, sodium propionate, and sodium butyrate) in an effort to maintain the fatty acids in the rumen-reticulum at normal levels and proportions and achieve a normal level of butterfat synthesis.

The study of milk of low fat content at Reading, England, has been recently extended by C. C. Balch and S. J. Rowland (*J. Dairy Res.* **26,** *162 (1959)*). They studied the action of sodium acetate, sodium propionate and sodium butyrate in animals fed a normal diet producing a normal level of butterfat as well as in Shorthorn cattle fed diets resulting in milk with a low fat content. Cattle fed a low hay diet for three weeks showed a significant decrease in the fat content of the milk, and the butterfat was further reduced with continued feeding of this diet. In most animals the butterfat content leveled out at a low percentage after 11 weeks on the diet.

These investigators determined the Reichert number for the fat in the milk and found good evidence for a relationship between fatty acid synthesis and the composition of butterfat. Acetate is absorbed directly from the reticulo-rumen and has been shown to be the source of part of the milk fat (G. Popják, T. H. French and S. J. Folley, *Biochem. J.* **48,** *411 (1951)*), and to be particularly active in the formation of fatty acids containing up to 16 carbon atoms. With lowered fat production in the milk there was a decrease in the Reichert number, while with the return to a normal butterfat content, the Reichert number increased, especially when sodium acetate was added to the diet.

Perhaps one of the most interesting aspects of this work by Balch and Rowland is the indication that acetate and butyrate may be important, but perhaps not the

controlling factors, in butterfat synthesis. When they added from 0.5 to 1.5 kg. of sodium acetate to the diet of cows in which the fat content of the milk had dropped from 4.8 per cent to a low of 2.8 per cent, there was a rapid (although not complete) recovery of the butterfat level. When sodium propionate was added there was no recovery. However, in one instance when sodium butyrate was added recovery was almost as good as with sodium acetate.

Although these investigators aimed at achieving adequate levels of acetate in the rumen-reticulum, the response to acetate indicated that this is only part of the mechanism required for normal butterfat production in dairy cattle. Further indication of this was the observation that the addition of fatty acids as sodium acetate, propionate or butyrate to normal roughage diets of cattle producing milk with a normal fat content had no effect upon either the fat content or the Reichert number of the milk.

Balch and Rowland also investigated the possibility that very succulent silage causes a reduction in butterfat content because of lack of fiber. Their results differed somewhat from those of Z. Zelter (*C. R. Acad. Sci. Paris* **231,** *1574 (1950)*) who suggested that the fibrousness of the silage may be a more controlling factor than the presence or absence of fatty acids in the silage.

While the work of Balch and Rowland was carried out on a comparatively small number of animals, their well-controlled investigation emphasizes the importance of the synthesis of fatty acids in the rumen in the production of milk as well as nonfatty solids. More importantly, however, their work emphasizes the fact that this is only a part of the overall metabolic picture respecting butterfat production. Further knowledge is needed concerning the mechanism whereby fiber in the diet results in normal fatty acid production, the importance of the proportion of different fatty acids, and other factors controlling butterfat synthesis.

DAIRY PRODUCTS AND DENTAL CARIES

Substitution of part of a cariogenic diet of weanling rats by various dairy products reduces tooth decay. Similar alterations of the maternal diet are without influence.

The influence of dietary carbohydrate in increasing the susceptibility of human subjects to dental caries was clearly shown by the Vipeholm study (*Nutrition Reviews* **12,** *233 (1954)*). Such a study, however, is laborious, expensive and time-consuming, and the development of a strain of caries-susceptible rats has aided materially in the study of the caries-initiating influence of various diets and foodstuffs, at least in a preliminary way (J. H. Shaw, *J. Nutrition* **41,** *13 (1950)*). It has been found, for example, that rats raised on a milk diet are resistant to the cariogenic influence of dissolved sugar but not dry sugar (*Nutrition Reviews* **13,** *316 (1955)*). It was not known, however, whether this influence of milk extended to the developmental period or was uniquely concerned with the posteruptive oral development.

In a study designed to clarify this point, J. H. Shaw, B. J. Ensfield and D. H. Wollman (*J. Nutrition* **67,** *253 (1959)*) carried out two experiments in which dairy products were substituted for a part of the cariogenic diet of either or both the maternal or filial generation of susceptible rats.

In the first experiment, 38 female rats were divided at weaning into four groups which were fed either the cariogenic diet alone or supplemented with whole milk, chocolate milk or chocolate drink (in which all but 2 per cent of the milk fat was removed) to provide, respectively, 30, 30 or

35 per cent of the daily caloric intake. After 24 weeks, they were bred to caries-susceptible males and the young of each group were maintained from weaning to 63 days on either the cariogenic diet or one of the dairy products, and from 63 to 121 days on the cariogenic diet alone or supplemented with one of the dairy products providing 33 per cent of the day's calories.

All animals in the maternal generation, although on different diets from the time of weaning, had similar growth and other gross characteristics. However, all those with the dairy supplements had significantly fewer caries, with plain milk appearing to be slightly more effective than chocolate milk, which was slightly more effective than chocolate drink. In the filial generation it was found that, regardless of the maternal diet, those rats on the dairy supplement from weaning again had significantly fewer caries than did those maintained on the caries-producing diet. Again, plain milk appeared to be slightly more effective than the other supplements.

In the second experiment, the maternal generation was allowed to have one litter while on the cariogenic diet. It was then divided into three groups of 15 rats each which were either fed the unsupplemented diet or given a 45 per cent caloric supplementation containing 66.3 per cent milk, 23.2 per cent vanilla ice cream and 10.5 per cent cheddar cheese, or the same combination with chocolate instead of plain milk. After three to four weeks on these diets, the rats were bred again and a second litter was produced. Both litters were divided into groups which, from weaning, were fed either the cariogenic diet alone or were fed the dairy products from 21 to 42 days and then transferred to the diet with the 33 per cent dairy supplement from 42 to 106 days.

In addition, some groups were placed on a 45 per cent supplemented diet from weaning to 42 days and a 25 per cent supplemented diet thereafter. Some of this generation were retained on their respective diets for a full 106 days after the 42 day diet change to allow for an exposure to the cariogenic diet for as long a period as those which had been on the unsupplemented diet from weaning.

It was noted that even in the maternal generation, in which exposure to the supplemented diets occurred after the first litter, there was a slight reduction in the incidence of dental caries. In the filial generation, dental caries was again reduced in all groups which had received the supplementation from weaning. Again, the maternal diet had little or no effect. An increase in caries was noted in those animals fed diets with less supplementation, but not in those which were studied for the longer period of time.

From these experiments, it is clear that a 33 per cent caloric supplementation of a cariogenic diet with various dairy products significantly reduces the incidence of tooth decay in rats. The period immediately after weaning appears to be especially important, since reduction of the 100 per cent supplementation to 45 per cent during this 21-day period resulted in a significant increase in caries in those animals on the plain milk supplementary mixture. The experiments did not, however, determine the nature of this post-developmental protection.

In every case, this effect was found only in the post-eruptive period and the diets of the mothers during the developmental period did not differ in their influence upon the caries activity of their progeny. Presumably this occurred because the diets fed the mothers during pregnancy and lactation were similar with respect to the nutrients required for tooth development and for establishment of caries-susceptibility. The authors point out that the cariogenic diet they used was a generous source of all known inorganic and organic nutrients and that the dairy products fed had no unique ability to promote teeth which were more caries-resistant. If the basal diet had been partially deficient or grossly unbalanced,

supplementation by the levels of dairy products could have resulted in a different situation.

These studies are especially intriguing because of their use of standard human dietary constituents for the prevention of tooth decay. There should be little difficulty in obtaining young human volunteers to subsist on a diet consisting largely of chocolate milk, ice cream and cheese. It would be important to ascertain whether replacement of 33 per cent of the cariogenic diet by other normal human foods will have the same effect.

DIETARY FAT AND HEPATIC LIPOGENESIS

Hepatic lipogenesis is found to be depressed by a high fat intake even when the carbohydrate supply is adequate.

One of the major factors influencing the synthesis of fatty acids by animal cells is the nutritional state of the animal. Definitive information on this subject was provided by G. E. Boxer and DeW. Stetten, Jr. (*J. Biol. Chem.* **153,** *607* (*1944*)), who found that undernourished rats displayed a very low rate of incorporation of deuterium into body fatty acids. Subsequent work by I. L. Chaikoff *et al.* (*J. Biol. Chem.* **185,** *845* (*1950*); *Ibid* **196,** *25* (*1952*)) demonstrated that when rats are fasted or fed a carbohydrate-free diet for a few days, the capacity of liver slices to convert the C^{14} of C^{14}-glucose or acetate-1-C^{14} to fatty acids *in vitro* is drastically reduced. Administration of a single dose of glucose, but not of protein or fat, completely restored hepatic lipogenesis to normal in the fasted rat.

The effect of fasting in reducing the lipogenic capacity of liver slices has been observed in experiments with intact rats injected with acetate-1-C^{14} (E. S. West *et al.*, *J. Biol. Chem.* **196,** *389* (*1952*)). A comparison of the lipogenic capacities of liver, gut, carcass and skin of intact adult rats fasted for various periods of time showed that the lipogenic mechanism in the liver was most susceptible to fasting (West *et al.*, *Ibid.* **208,** *115* (*1954*)).

The capacity of the liver for lipogenesis is also regulated by hormones (R. O. Brady *et al.*, *J. Biol. Chem.* **193,** *459* (*1951*)), particularly insulin (Chaikoff *et al.*, *J. Biol. Chem.* **193,** *549, 557* (*1951*)). The effect of insulin is considered to be secondary to its action in priming glucose utilization (Chaikoff *et al.*, *J. Biol. Chem.* **197,** *621* (*1952*)).

R. Hill, J. M. Linazasoro, F. Chevallier and Chaikoff (*J. Biol. Chem.* **233,** *305* (*1958*)) have also demonstrated an influence of the amount of fat ingested by the rat on hepatic lipogenesis. In one experiment, rats were fed synthetic diets containing 55 per cent of glucose, 22 per cent of casein and 0, 1, 2.5, 5, 10 or 15 per cent of corn oil. Each change in the percentage of fat in the diet was made at the expense of cellu-flour. The diets were fed for only three days before the animals were sacrificed so that the possibility of appreciably altering liver composition by the prolonged feeding of high fat diets was minimized. The average daily food intakes of the rats were similar, but no attempt was made to keep the caloric value per gram of diet constant.

As the percentage of corn oil in the diet was increased from 0 to 10, the percentage conversion of added acetate-1-C^{14} to fatty acids by liver slices decreased from 20 to about 3.0. No further fall in the extent of conversion occurred in liver slices from rats fed a diet containing 15 per cent of corn oil.

In another experiment, the caloric value per gram of diet was kept from varying greatly by lowering the level of casein in the diet from 37 to 15 per cent as the amount of corn oil was increased from 0 to 15 per cent. The glucose content of these diets was 50 per cent. As before, the average

daily food intakes of rats on these diets were similar. Here again the incorporation of acetate-1-C^{14} as well as that of C^{14}-glucose into fatty acids was decreased as the fat concentration in the diet was raised.

To show that these results were not influenced by the differences in casein intake, another experiment was carried out in which rats were fed synthetic diets containing no fat and either 15 or 37 per cent of casein. No difference was observed in the capacity of liver slices from these two groups of rats to incorporate acetate-1-C^{14} into fatty acids. Also, to show that the effect was not specific for corn oil, a vegetable oil, a hydrogenated vegetable oil and lard were tested. Each was as effective as corn oil in decreasing hepatic lipogenesis from acetate carbon.

In all of these experiments the percentages of added acetate-1-C^{14} recovered as $C^{14}O_2$ did not differ significantly from group to group. This, in conjunction with the observation that feeding extra fat did not depress the total utilization of acetate by the liver slices (as estimated by measuring the C^{14} remaining in the medium at the end of the incubation period), suggested that the ingestion of fat did not result in a general depression of the metabolic activity of the liver, but rather in a specific inhibition of lipogenesis.

The effects of high levels of fat in the diet on the lipogenic capacity of the liver were also studied in experiments with intact rats. The animals were fed isocaloric diets containing 0, 5, 10, or 15 per cent of corn oil, for three days. They were then injected with acetate-1-C^{14} and killed one hour later. The livers were removed and analyzed for C^{14}-fatty acids. Again the percentage of C^{14}-fatty acids recovered from the liver decreased as the amount of fat in the diet increased.

These effects of ingested fat upon hepatic lipogenesis occurred without changes in the glycogen and fat content of the liver or in the glucose and lipid content of plasma. Also, in no case was there a decrease in the glycogen content of the liver of rats fed the high fat diets for three days. "Thus, the depressing effect of dietary fat upon hepatic lipogenesis was observed under conditions that allowed for priming of hepatic lipogenesis by carbohydrate."

In view of the influence of insulin on hepatic lipogenesis, the possibility that the depression in lipogenesis produced by a high fat diet might be a result of a relative decrease in available hormone was examined. In normal rats fed on diets containing 50 per cent of glucose, with or without added fat, the administration of two units of insulin twice daily for three days failed to prevent the depression in hepatic lipogenesis induced by a high fat intake.

The results of these experiments indicate that the lipogenic activity of the liver is controlled in some way by the amount of fat ingested and that hepatic lipogenesis is depressed by a high fat intake, even when the diet contains a high percentage of carbohydrate. The mechanism of this adaptive response remains to be explained, but it does not appear to be mediated by the depressing effect of a high-fat intake on insulin production.

AN ENZYMATIC FUNCTION OF CARNITINE

With palmitate (but not shorter chain fatty acids) the addition of carnitine to rat liver homogenates increases the formation of ketone bodies but has no influence on carbon dioxide production.

The recognition that carnitine was required for the growth of the meal worm (*Nutrition Reviews* **13,** *24 (1955)*) stimulated work to determine the metabolic role of this compound. Although only very small amounts of carnitine are required for

the growth of the meal worm, the tissues of higher animals contain fairly large amounts of it. Skeletal muscle contains the highest concentration of carnitine (560 to 1120 micrograms per gram).

G. Fraenkel (*Nutrition Reviews, loc. cit.*) showed that carnitine was in the developing chick, appearing fairly early and increasing in content as the embryo matured. No carnitine could be detected in the unfertilized egg.

During the past few years I. B. Fritz has investigated the role of carnitine in the intact rat and in enzymatic preparations of livers removed from these animals. He was led to this work by E. Lundsgaard, who found evidence for a factor in blood and muscle extract which increased the oxygen consumption of perfused cat livers (*Biochim. Biophys. Acta* **4,** *322 (1950)*).

Fritz (*Acta Physiol. Scandinav.* **34,** *367 (1955)*) used liver slices and homogenate preparations instead of the perfused whole liver. He found that when either labeled glucose, acetate or palmitate was the substrate, the production of labeled ketone bodies was increased by the factor in blood and muscle extract. Carnitine could partially replace the blood or muscle extract.

Later Fritz was intrigued by the observation of C. Artom (*J. Biol. Chem.* **213,** *681 (1955)*) that the livers of choline-deficient rats oxidized labeled fatty acids at a slower rate than did similar preparations from normal animals. The similarity in the structure of choline and carnitine suggested to Fritz that choline might be a precursor of carnitine or a carnitine derivative.

To test the above hypothesis, he put weanling rats on choline-deficient diets containing either 5 or 20 per cent casein (*Am. J. Physiol.* **190,** *449 (1957)*). The livers from the choline-deficient animals were homogenized and added to a buffer containing inorganic salts, adenosine triphosphate and palmitic-1-C^{14} acid. Carnitine at a concentration of 6.7×10^{-5} molar was added to half of the Warburg flasks. The presence of this amount of carnitine (30 micrograms in 4.5 ml. of medium) increased the formation of ketone bodies over that seen in the control flasks without the added carnitine (513 counts versus 214 counts per minute per mg. of dry fat-free liver). The carnitine had no effect on the production of carbon dioxide in this system. Livers from the control rats fed a commercial stock diet as well as those from the choline-supplemented animals showed a similar response.

The above observations led Fritz to conclude that choline is not converted to carnitine or a carnitine derivative.

Another observation made by Fritz (*loc. cit.*) was that the liver homogenates from the choline-supplemented rats showed a greater carbon dioxide production than those from the deficient rats. In these experiments, labeled palmitic acid was also the substrate. Since the homogenates from the livers of both the supplemented and deficient rats still responded to the addition of carnitine with an increase in ketone production, Fritz suggested that choline and carnitine "influence different parameters of fat metabolism."

In an accompanying paper, I. B. Fritz and P. DuPont (*Am. J. Physiol.* **190,** *453 (1957)*) reported that carnitine could not replace choline as a lipotropic factor. In these studies they used weanling rats maintained on a choline-deficient diet containing 20 per cent protein. On this diet, the rats developed livers containing 16 per cent fat. In another group of rats the addition of 2.6 g. of choline chloride per kg. of diet reduced the fat level of the livers to 5.3 per cent. The addition of 3.4 g. of DL-carnitine chloride to one kg. of the choline-deficient diet resulted in fat levels of 13.8 per cent in the livers of a third group of rats.

In an attempt to elucidate the mechanism whereby carnitine increased the ketone body production of liver homogenates, Fritz carried out a variety of enzymatic studies (*Am. J. Physiol.* **197,** *297 (1959)*). He prepared liver slices from male rats

weighing about 200 g. and incubated them with inorganic salts. To half of the Warburg vessels, carnitine was added at a level of 3×10^{-4} molar. When labeled octanoate was present in the flasks, there was no increase in the formation of carbon dioxide or of ketone bodies. However, when either palmitate or stearate, both labeled in the carboxyl group, were incubated with the slices, there was an increased ketone production (110 to 140 per cent over that of the controls) but no change in the carbon dioxide production.

He prepared a liver particulate fraction which represented 25 per cent of the original liver weight. When this was incubated with labeled butyrate, octanoate or laurate, there was no effect of carnitine addition on the production of carbon dioxide or the formation of ketones. Again, in the presence of palmitate or stearate, carnitine increased the formation of ketones by a factor of 2 to 4.

The effect of carnitine in increasing the formation of ketone bodies by liver preparations incubated with palmitic acid was seen at several different levels that were roughly comparable to those which might exist in serum, if all the unesterified fatty acids were present as palmitate (10^{-4} to 10^{-3} molar). When the level of palmitate was held constant, there was a direct relation between the formation of ketone bodies and the amount of carnitine added to the Warburg flask. The relationship was essentially linear over the range from 1.0 to 100.0 micrograms.

The observed effect of carnitine was not related to the formation of palmityl-coenzyme A. This was shown by the observation that carnitine did not enhance the formation of ketone bodies when the palmityl-coenzyme A was incubated with rat liver particulates. The preceding finding suggested that carnitine probably influenced some reaction prior to the formation of palmityl-coenzyme A. To test this possibility, enzymatic studies were carried out with hydroxylamine which traps the activated fatty acids (*i.e.*, as the coenzyme A complex). The addition of carnitine was without effect on the amount of palmityl hydroxamate formed. Fritz was led to conclude that "carnitine influences long chain fatty acid oxidation at a level prior to the formation of the coenzyme A derivative in a system which retains some semblance of intracellular structure."

Whether the effect of carnitine in increasing the formation of ketone bodies from long chain fatty acids is an artifact or represents an important physiological function will remain for future research. The observations of Fritz provide an important clue and suggest strongly that carnitine may be important in some phase of the metabolism of long chain fatty acids.

MOLYBDENUM TOXICITY

It is possible that molybdenum exerts its toxic action by virtue of an effect on certain enzyme systems rather than by a direct effect on mineral and chondroitin sulfate deposition in the bones.

Cattle, rabbits and chicks fed high dietary levels of molybdenum all show symptoms that include deformities of the joints of extremities, reduction in the rates of growth, anemia and diarrhea (W. S. Ferguson *et al.*, *J. Agr. Sci.* **33,** *40 (1943)*; L. R. Arrington and G. K. Davis, *J. Nutrition* **51,** *295 (1953)*; and B. L. Reid *et al.*, *Fed. Proc.* **17,** *294 (1958)*). The bone deformity is strongly suggestive of a disturbance in calcium and

phosphorus metabolism, but R. F. Miller and N. O. Price (*Fed. Proc.* **15,** *564 (1956)*) have reported that the addition of inorganic sulfate to high molybdenum diets alleviates the growth depressing effects of the molybdenum. Thus, the deformities were suspected to be due to an imposed failure of the organic phase of bone metabolism.

In order to resolve this question, J. P. Feaster and G. K. Davis (*J. Nutrition* **67,** *319, 325 (1959)*) have carried out nutritional experiments on growing rabbits employing radioactive sulfate, calcium and phosphorus.

In the studies treating calcium and phosphorus metabolism, one group of rabbits was fed a control diet of rolled oats (88 per cent) alfalfa meal (10 per cent) and sucrose (2 per cent), with calcium acetate added to furnish the proper calcium to phosphorus ratios. The second group received the same basal diet with molybdenum added at a level of 0.2 per cent as sodium molybdate. After the animals had been fed these diets for five weeks, each received a single oral dose containing 3 microcuries of Ca^{45} and P^{32}.

Three rabbits from each lot were killed after intervals of six, 24 and 48 hours and seven and 14 days. Blood, liver, kidney, skeletal muscle, cardiac muscle and bone were taken for determinations of both radioactive and stable calcium and phosphorus. Urine and feces collected at 24, 48, 96 and 168 hours after administration of the radioactive materials were also analyzed for calcium and phosphorus.

Although food consumption by the rabbits receiving molybdenum was slightly lower than that of the control rabbits, the difference in weights of the animals at sacrifice could be only partially accounted for on this basis. All symptoms of molybdenum toxicity as mentioned above were present.

There was no appreciable effect of the level of molybdenum in the diet on uptake of radioactive calcium and phosphorus in the soft tissues. Amounts of Ca^{45} and P^{32} excreted by the control rabbits exceeded only slightly the amounts excreted by the high-molybdenum rabbits, except in the fecal excretion of P^{32}. Rabbits of the control group also excreted more total calcium and phosphorus in both urine and feces than those of the high-molybdenum group, because of higher feed consumption by the control animals.

In view of the skeletal deformities observed in the rabbits on high-molybdenum intake, it was expected that bone would show the greatest alterations in calcium and phosphorus content. Decreased contents of stable calcium and phosphorus and decreased uptake of Ca^{45} and P^{32} were anticipated. Nevertheless, analyses of the humeri did not bear out this prediction. The uptake of Ca^{45} was slightly greater in the humeri of the high-molybdenum rabbits than in the controls and apparently continued over a longer period of time. While the uptake of P^{32} was less rapid in the humeri of the high-molybdenum rabbits during the first six hours after administration, P^{32} levels in these animals exceeded control levels between 24 and 48 hours after administration of the isotopes and at 14 days were nearly twice as high as control P^{32} values. Absolute amounts, as well as concentrations, of Ca^{45} and P^{32} were higher in the humeri of the high-molybdenum rabbits than in the controls. Thus, an increased uptake of these elements was seen to have occurred following ingestion of relatively large amounts of molybdenum.

Weights of humeri from the high-molybdenum animals sacrificed two weeks after isotope administration averaged 2.92 g. as compared with 5.48 g. in control animals. Yet, the total amounts of calcium and phosphorus present in the smaller bones from the molybdenum-treated rabbits were nearly as high as those in control bones. It is, therefore, presumed that the decrease in weight of the humeri was the result of diminution of organic matter. Part of this

was probably due to decreases in marrow since anemia is a common result of molybdenum toxicity. The authors report that H. F. Roberts (*Ph.D. Dissertation, University of Florida, 1956*), had observed areas of necrosis in the marrow and a decrease in the number of nucleated red blood cells in the blood sinuses.

Although Roberts (*loc. cit.*) had noted that there was no evidence of defective deposition of inorganic materials in the cartilage matrix, Feaster and Davis carried out a series of studies to determine what effect a high level of molybdenum in the diet has on the tissue distribution and excretion of radioactive sulfate. The rabbits were fed control and experimental diets similar to those that had provided the data on calcium and phosphorus metabolism. After the rabbits had been on these diets for five weeks, each received a single oral dose of 10 microcuries of S^{35} in the form of sodium sulfate. Tissues taken for determinations of both radioactive and stable sulfur included liver, muscle, bone and hair. Urine and feces were also analyzed.

Levels of S^{35} found in the livers and bones of these rabbits were lower than in rabbits maintained on the control diet. Urinary excretion of S^{35} was higher, indicating decreased retention of sulfur in the high-molybdenum animals. Yet, in the humeri, alone, total sulfur content was significantly higher than in control rabbits, indicating a higher proportion of cartilage. (Most of the sulfur present in the skeleton occurs in the cartilage as chondroitin sulfate.)

Because sulfur usually serves as an indicator of protein metabolism, it is possible that the excretion of a higher amount of urinary sulfur indicated a lowered protein metabolism in molybdenum fed animals. Analyses of the urines for nitrogen revealed an average excretion of 27 mg. per control rabbit, compared with 63 mg. for the high-molybdenum rabbits. The authors are of the opinion that if the control rabbits are considered to be in nitrogen balance, or even slightly on the positive side since they were in the growing stage, then the high-molybdenum rabbits were probably in negative nitrogen balance. In wasting accompanying starvation, the urinary nitrogen to sulfur ratio remains virtually unchanged. However, in the present study the nitrogen to sulfur ratio of the high molybdenum rabbits was twice as high as that of the controls, indicating toxic effects of the high intake of molybdenum over and above those due to decreased food intake.

According to these investigators, it is possible that molybdenum exerts its toxic action by virtue of an effect on certain enzyme systems. M. Bossard (*Bull. Soc. Chim. Biol.* **29,** *218* (*1947*)) noted that sodium molybdate inhibited all phosphatases found in extracts of plant tissues. On the other hand, D. A. Richert and W. W. Westerfeld (*J. Biol. Chem.* **203,** *915* (*1953*)) have isolated from soy flour a molybdenum salt essential to the deposition and maintenance of normal levels of rat intestinal xanthine oxidase. Thus, it appears that molybdenum, up to certain concentrations, may be necessary to the action of certain enzymes, but beyond these levels may act as inhibitors of these or other enzymes.

DIETARY FATTY ACID INTERRELATIONSHIPS

Essential fatty acids are required for proper utilization of fat calories. High ratios of saturated fats may promote essential fatty acid deficiency symptoms.

Although nutritionists have been concerned for many years with the optimum amount and composition of fat for the human diet, the most that can be said with

any certainty at the present time is that the optimum lies between the limits of the very small amount necessary to supply sufficient essential fatty acids and the excessive amounts which appear to lead to hyperlipemia and, possibly, to atherosclerosis. Aside from the question of adequate essential fatty acid intake, but inevitably tied to the problem of total dietary fat, is that of the optimum proportion of saturated and unsaturated fat. Some consideration has been given to this problem (*Nutrition Reviews* **14,** *305* (*1956*)) but the result has been only a start in the right direction.

To further complicate the entire picture, it may be recalled that Greenberg and coworkers found that, although male rats which had been on a fat-free diet from weaning maintained a fairly normal growth rate when given a supplement of 100 mg. of methyl linoleate per day, they grew even faster when this supplement was given in cottonseed oil (S. M. Greenberg *et al.*, *J. Nutrition* **41,** *473* (*1950*); **45,** *521* (*1951*)). Evidently, either the cottonseed oil had additional growth promoting factors or it acted in a complementary manner with the linoleate. It should be stressed, however, that growth rate may not be a sufficient criterion for dietary excellence (*Nutrition Reviews* **16,** *350* (*1958*)).

The relationship between the essential fatty acid content of the diet and total saturated fat has recently been studied by J. G. Peifer and R. T. Holman (*J. Nutrition* **68,** *155* (*1959*)). Two separate experiments were performed. In the first, hydrogenated coconut oil and ethyl esters from corn oil were fed to male weanling rats in various proportions as part of an otherwise adequate diet. With 1 per cent corn oil and 9 per cent hydrogenated coconut oil, growth was significantly better than with 1 per cent of either hydrogenated coconut oil or corn oil or with 10 per cent hydrogenated coconut oil. Dermal symptoms, which were also studied, were absent in both groups fed corn oil, but very significant in its absence.

In the second experiment, various proportions of hydrogenated coconut oil and ethyl linoleate up to a total of 25 per cent of the diet weight (or 47.6 per cent of the calories) were fed. In this case, it was observed that in the absence of ethyl linoleate, growth rates were decreased when the hydrogenated coconut oil was increased from 1 to 25 per cent. Dermal symptoms, however, decreased on increasing the coconut oil (perhaps as a result of the decreased growth rate). When 0.5 per cent ethyl linoleate was supplied, however, 24.5 (but not 9.5 per cent) hydrogenated coconut oil appeared to be somewhat growth inhibiting. Caloric efficiencies were considerably lower for rats on the essential fatty acid deficient diets and varied inversely with the coconut oil content.

Analysis of the polyunsaturated acids of the hearts of animals in this experiment revealed that as the hydrogenated coconut oil content of the diet was increased, the heart polyunsaturated acids of all types increased. Since, in half the rats no linoleate was being ingested, the source of this acid and the higher polyunsaturated acids elaborated from it must have been in the other tissues. This point, however, was not investigated in these studies. In particular, trienoic acids, which are known to accumulate in the fat deficiency state (*Nutrition Reviews* **15,** *86* (*1957*)), were very high in the animals on the ethyl linoleate-deficient diets. With the linoleate-containing diets, increasing the hydrogenated coconut oil content to 24.5 per cent resulted in a manifold increase in the trienoic acid (to about 30 per cent of the values for linoleate-deficient diets). This suggested that a change in the direction of essential fatty acid deficiency had been produced. Tetraenoic, pentaenoic and hexaenoic acids tended to increase on the diets high in hydrogenated coconut oil, but, unlike the trienoic acid, the values were generally greater when the diet contained ethyl linoleate.

In general, although the total endogenous polyunsaturated acids (all the above minus linoleate) tended to increase in the hearts with an increase in the dietary saturated fat, the relative amounts of these acids remained fairly constant. The exception was the increase in the relative amounts of trienoic acid in the rats fed ethyl linoleate with increasing hydrogenated coconut oil. This points toward a possible competitive dehydrogenation of the saturated fatty acids which were fed in increasing proportions, producing eicosatrienoic acids from the dehydrogenation of palmitic and stearic acids.

The authors conclude that with an adequate intake of essential fatty acids, increased dietary saturated fat stimulates growth rate. In essential fatty acid deficient animals, however, the saturated fat is not used for growth. It appears, then, that the essential fatty acids are in some way necessary for proper utilization of the saturated fatty acids. Three possible reasons were suggested as to why increased dietary saturated fat stimulated an increased polyunsaturated fatty acid content in the heart. These were (1) a sparing action of saturated fat on the catabolism of heart polyunsaturated acids, (2) a mobilization of polyunsaturated acids to the heart, and (3) a stimulating effect of high levels of dietary saturated fat on polyunsaturated fatty acid synthesis. In this latter consideration it is possible that the tetraenoic acid synthesized by the ethyl linoleate-deficient animals may not be arachidonic.

These considerations lead to the conclusion that the fat content of the diet must be studied with respect to the ratios of the different types of fatty acids present and their interactions with each other. It will be important now to study the relationships of the mono-unsaturated acids, oleic and palmitoleic, the most prevalent class of dietary fatty acids, which may not behave like either the saturated or polyunsaturated classes.

METHIONINE METABOLISM

The substitution or supplementation of methionine with choline, folacin, homocystine and vitamin B_{12} in the diet of the mouse has relatively little effect.

Numerous studies in experimental animals, including the rat, chick and other species, have established the existence of relationships among methionine, choline, folic acid and vitamin B_{12} (A. E. Schaefer and J. L. Knowles, *Proc. Soc. Exp. Biol. Med.* **77,** *655 (1951)*; M. A. Bennett, *J. Biol. Chem.* **187,** *751 (1950)*; J. A. Stekol *et al.*, *Arch. Biochem. Biophys.* **36,** *5 (1952)*; T. H. Jukes and E. L. R. Stokstad, *J. Nutrition* **48,** *209 (1952)*). These relationships apparently involve the synthesis, transfer and utilization of methyl groups.

Relatively few studies of these relationships have been carried out in mice. E. Nielsen and A. Black (*J. Nutrition* **28,** *203 (1944)*) have obtained some evidence that the mouse requires folacin for growth and reproduction. Anti-folacin compounds produce growth depressions in mice that may be reversed with folacin or leucovorin (H. P. Broquist *et al.*, *J. Nutrition* **47,** *93 (1952)*).

In conjunction with the use of thyroid-active substances or high protein diets, vitamin B_{12} has been shown to stimulate the growth of mice (D. K. Bosshardt *et al.*, *J. Nutrition* **40,** *595 (1950)*). Y. C. P. Lee *et al.* (*Am. J. Physiol.* **173,** *456 (1953)*) reported that fetal resorption in the mouse could be prevented with either alpha-tocopherol or vitamin B_{12} supplementation.

Since several investigations had indicated that the mouse was more resistant to a choline deficiency than the rat or chick

(H. M. Barret *et al.*, *J. Physiol.* **93,** *367 (1938)*), H. E. Sauberlich (*J. Nutrition* **68,** *141 (1959)*) has made a concentrated study of the possible relationships of methionine, choline, folacin and vitamin B_{12} in the mouse.

Commercial weanling male albino mice of the Rockland Swiss-Webster strain were given food and water ad libitum and weighed twice a week. A methionine- and methyl-free basal diet was used in nearly all experiments consisting of 12 per cent oxidized casein or methanol-extracted casein, 4 per cent corn oil, 1 per cent cod liver oil, 4 per cent salts, 72.5 per cent sucrose, 0.3 per cent L-cystine, and 0.2 per cent DL-tryptophan. Adequate amounts of inositol, calcium pantothenate, alpha-tocopherol, alpha-tocopherol acetate, niacin, riboflavin, pyridoxine, thiamine, 2-methyl-1, 4-napthoquinone and biotin were added routinely, and supplements of varying amounts of choline, homocystine, betaine, folacin, vitamin B_{12}, methionine and other amino acids constituted the experimental procedure. In some of the animals, fat content and the transmethylase and choline oxidase activities of the liver were determined.

Mice fed a methionine-free, choline-free basal diet, supplemented with homocystine, died or failed to grow when either folacin or vitamin B_{12} was added alone. The addition of both vitamins permitted survival but resulted in little growth. Further supplementation with glycine, serine and threonine resulted in growth about 50 per cent of normal.

When choline alone was added to the homocystine-supplemented basal diet, only slight growth resulted. On the other hand, about 50 per cent of normal growth was achieved with betaine. When folacin was added in either of the situations cited above, nearly normal growth resulted and the further addition of vitamin B_{12} had only a slight effect. Methionine alone permitted nearly normal growth, which was improved slightly by the addition of choline, folacin and vitamin B_{12}.

The observation that homocystine, in the absence of a methyl source, required both folacin and vitamin B_{12} to permit survival and limited growth would indicate that these vitamins are involved in the synthesis, transfer or utilization of methyl groups. Studies with other species indicate that vitamin B_{12} aids in the synthesis of methyl groups, while folacin is involved in certain transfers of methyl groups.

Apparently the requirement for folacin in the methylation of homocysteine is reduced when betaine is the source of methyl groups rather than choline. The author explains this on the basis that the requirement for folacin in order to maintain choline oxidase activity in the conversion of choline to betaine, as demonstrated in the rat and the chick, would be avoided. Folacin may still be required for the betaine-homocystine transmethylase reaction.

Sauberlich found that liver choline oxidase activity was markedly reduced in mice fed the basal diet supplemented with homocystine alone. The reduction in activity was largely prevented by dietary supplementation with folacin and, in part, by suplementation with vitamin B_{12}. Choline was also effective but betaine was not.

Transmethylase activity of the liver was reduced when choline, folacin and vitamin B_{12} were omitted from the basal homocystine diet. When folacin and vitamin B_{12} were added to the diet the activity was improved. Choline and folacin added together gave normal transmethylase activity, while only partial improvement was noted following addition of choline and vitamin B_{12}. Thus the importance for the mouse of vitamin B_{12} in relation to this activity is not certain. The effect of vitamin B_{12} may be a reflection of needs for methyl groups that could be furnished by *de novo* synthesis in the presense of vitamin B_{12} but could not be fur-

nished by choline except in the presence of folacin.

The author noted that extremely fatty livers were produced in mice fed the basal homocystine diet, either in the presence or absence of folacin and vitamin B_{12}. The presence of choline or betaine largely prevented this condition, and supplementation with choline, folacin and vitamin B_{12} resulted in normal liver fat levels.

RIBOFLAVIN REQUIREMENTS OF THE CAT

In the cat, as in man, the riboflavin requirement is increased by a high-fat diet and decreased by a high-carbohydrate diet.

Little is known of the nutritional requirements of the domestic cat. In an initial attempt to close this hiatus in our knowledge of the nutrition of laboratory animals, S. N. Gershoff, S. B. Andrus and D. M. Hegsted (*J. Nutrition* **68,** *75 (1959)*) have studied the riboflavin requirements of cats fed diets varying in carbohydrate and fat content.

Three- to six-months-old kittens of mixed breed and sex were used. The two diets employed in the studies differed considerably in their fat and carbohydrate content. In the high-fat diet 46 per cent of the calories came from fat and 29 per cent from carbohydrates, and in the high-carbohydrate diet 11 per cent of the calories were from fat and 64 per cent from carbohydrates. In both diets, 25 per cent of the calories were provided by casein.

Two separate experiments were carried out. In the first, nine kittens were fed the low-carbohydrate, high-fat diet without riboflavin for two months, by which time all were suffering from riboflavin deficiency. Four cats were sacrified for histological study. The others were given 5 mg. of riboflavin subcutaneously and then fed diets with graded amounts of riboflavin. Two cats received 0.5 mg., two cats 1.0 mg., and one cat 1.5 mg. of riboflavin per kg. of diet. All of these cats died, presumably of riboflavin deficiency, within four months of starting these diets and were autopsied within two hours following death.

In a second experiment, cats were fed both experimental diets with varying amounts of riboflavin for periods up to 34 months. Acute deficiency in cats fed diets without added riboflavin resulted in anorexia accompanied by weight loss terminating in death. In the initial experiment, seven of nine cats showed some loss of hair about the eyes and ears after two months of the unsupplemented diet.

In acute riboflavin deficiency, those animals fed the high-fat diet became moribund sooner than those fed the high-carbohydrate diet. Chronic riboflavin deficiency differed from acute deficiency not only in a more protracted course but also in the occurrence of cataracts. All five animals that developed cataracts were receiving the high-fat diet. These cataracts occurred some months after the vitamin-deficient diet was started. In none of the cats in either experiment were there lesions of the cornea, changes about the mouth, anemia, paralysis, or the riboflavin collapse syndrome.

Fat was generally present in the parenchymal cells of the livers of deficient cats. All the male cats, whose testes were studied histologically, showed distinct testicular hypoplasia. There was generally complete aspermia and decreased numbers of spermatids and spermatocytes. No alterations of the myelin structures of the brain or spinal cord were observed. Skin changes were less dramatic than for other species.

Two cats remained in excellent health for more than 34 months on the high-carbohydrate diet supplemented with 3 mg. of riboflavin per kg. Cats were routinely

maintained in the laboratory on the low-carbohydrate diet plus 4 mg. of riboflavin per kg. for periods up to two years.

Urinary and fecal riboflavin analyses revealed that the better maintenance on the high-carbohydrate diet was caused by greater synthesis of riboflavin in these animals. Since synthesis was high on both diets, the authors concluded that the effect was due to both increased intestinal synthesis and utilization.

These results are in contrast to observations of R. L. Potter *et al.* (*J. Nutrition* **24,** *449 (1942)*) on dogs, and of G. J. Mannering *et al.* (*Proc. Soc. Exp. Biol. Med.*, **46,** *100 (1941)*) on rats. None of the latter workers was able to detect a sparing action of sucrose on the dietary requirements for riboflavin. Riboflavin deficiency has, however, been associated with cataracts in the rat (P. L. Day and W. C. Langston, *J. Nutrition* **7,** *97 (1934)*), the mouse (S. W. Lippincott and H. P. Morris, *J. Nat. Cancer Inst.* **2,** *601 (1941)*) and the pig (A. J. Patek, Jr., *et al.*, *Am. J. Physiol.* **133,** *47 (1941)*).

The actual incidence of cataract production in the rat is highly variable, ranging from 0 to 100 per cent. Fatty livers have been described in riboflavin-deficient dogs, although one of the groups studying this phase of riboflavin deficiency (H. R. Street *et al.*, *J. Nutrition* **22,** *7 (1941)*) concluded after further work that such changes could be explained on the basis of inanition. Partial paralysis because of myelin degeneration of the control nervous system and peripheral nerves, so prominent in other species (H. M. Zimmerman and E. Burack, *J. Exp. Med.* **59,** *21 (1934)*; J. H. Shaw and P. H. Phillips, *J. Nutrition*, **22,** *345 (1941)*; L. D. Greenberg and J. F. Rinehart, *Fed. Proc.* **15,** *265 (1956)*) were not observed in riboflavin-deficient cats.

B. Bro-Rasmussen (*Nutrition Abst. Rev.* **28,** *1, 369 (1958)*) has summarized and evaluated much of the data available on the riboflavin requirements of different species and has concluded that the minimal requirement of different species is the same if expressed upon a calorie basis. The minimum need of adult man is, by this yardstick, concluded to be approximately 0.25 mg. per 1000 calories, and 0.5 to 0.6 mg. per 1000 calories is assumed to be an adequate intake. Bro-Rasmussen also concludes that the intestinal flora may be of importance for man's riboflavin supply.

Gershoff, Andrus and Hegsted (*loc. cit.*) also concluded that the studies of E. M. Widdowson and R. A. McCance (*Medical Research Council Special Report Series, No. 287 London, 1954*) in German orphanages provide substantial evidence that the intestinal synthesis and the urinary excretion of riboflavin depend on the ratio of fat to carbohydrate calories in the diet.

UTILIZATION OF HYDROXYANTHRANILATE

The evidence presented supports the view that 3-hydroxyanthranilic acid is an intermediate in the conversion of tryptophan to niacin in guinea pigs, chicks and hamsters.

It is well known that animal species differ in their dietary requirement for niacin. These differences probably result in large measure from the fact that tryptophan substitutes for this vitamin in varying degrees in different species (W. A. Krehl *et al.*, *Science* **101,** *283, 489 (1945)*). The general pattern of biosynthesis of niacin from tryptophan by *Neurospora crassa* has been well established, and there is abundant evidence that this pathway occurs in mammals. H. K. Mitchell and J. F. Nyc (*Proc. Nat. Acad. Sci.* **34,** *1 (1948)*) have identified 3-hydroxyanthranilic acid as an intermediate in this conversion in studies with *Neurospora*. Its niacin-replacing activity in the rat is

approximately equal to that of tryptophan or about 2 per cent of that of niacin (Mitchell *et al.*, *J. Biol. Chem.* **175,** *433 (1948)*).

D. H. Decker and L. M. Henderson (*J. Nutrition* **68,** *17 (1959)*) point out, however, that *in vitro* studies, chiefly with rat tissues, have not confirmed observations made in the intact rat that oxidation of 3-hydroxyanthranilate by slices and cell-free preparations has failed to produce niacin (A. H. Bokman and B. S. Schweigert, *Arch. Biochem. Biophys.* **33,** *270 (1951)*; Henderson and H. M. Hirsch, *J. Biol. Chem.* **181,** *667 (1949)*; A. H. Mehler, *Ibid.* **218,** *241 (1956)*).

However, according to R. J. Suhbadolnik *et al.* (*J. Biol. Chem.* **228,** *973 (1957)*) the liver filtrates from ten different mammalian species varied considerably in the rate at which they oxidized 3-hydroxyanthranilic acid, the liver of the rat containing less of the 3-hydroxyanthranilic acid oxidase than most of the species studied.

Decker and Henderson (*loc. cit.*) therefore decided that the results of *in vitro* studies must be checked by carrying out determinations *in vivo* on each species that had been studied *in vitro*. In an initial growth study, three species, the chick, the guinea pig and the hamster, were characterized with respect to their efficiency in substituting 3-hydroxyanthranilate for niacin.

Since so many species appear to utilize tryptophan as a source of niacin, the diets were prepared so as to make this the limiting amino acid. All amino acids, except tryptophan, were provided at levels in excess of the minimum requirement by the addition of proteins low in tryptophan such as corn proteins and gelatin.

Data presented by G. M. Briggs *et al.* (*Proc. Soc. Exp. Biol. Med.* **51,** *59 (1942)*) indicated that the efficiency of conversion of tryptophan in the chick is about 4 per cent (the chick could grow about as well on a niacin-free diet supplemented with 100 mg. per cent tryptophan as on the same diet supplemented with 2.5 mg. per cent niacin). The level of 3-hydroxyanthranilic acid selected by Decker and Henderson for their experiments was 80 mg. per cent. The results indicated that while 3-hydroxyanthranilic acid is used to replace niacin, it is not as effective at 80 mg. per cent as niacin is at 2.5 mg. per cent. Thus, according to these experiments, 3-hydroxyanthranilate has less than 4 per cent of the effectiveness of niacin in supporting growth in chicks.

Their results with the guinea pig agreed well with those appearing in the literature (M. D. Cannon *et al.*, *Proc Soc. Exp. Biol. Med.* **63,** *414 (1946)*). When 100, 200 and 300 mg. per cent of 3-hydroxyanthranilic acid were fed, Decker and Henderson found that 300 mg. per cent gave better growth than either of the two lower levels and was also more effective than 3 mg. per cent of niacin. They point out that 3-hydroxyanthranilic acid has a niacin-replacing activity in the guinea pig approximately equal to that of tryptophan.

In preliminary experiments, three levels of 3-hydroxyanthranilic acid were tested in hamsters. Seventy-five mg. per cent gave better growth than two lower levels and supported growth comparable to that obtained with 1.5 mg. of niacin. The authors report that the hamsters were markedly affected by niacin deficiency. During the first 12 days of the growth studies, the weights of the animals in all three groups were about the same, but by the end of the second week losses of weight were observed in all the negative control animals. Two died on the eighteenth day and two others developed diarrhea and unkempt fur.

The authors conclude that 3-hydroxyanthranilate has approximately the same niacin-replacing activity as tryptophan in the chick, guinea pig and hamster. They are of the opinion that the evidence presented supports the view that in these species 3-hydroxyanthranilic acid is an intermediate in the conversion of tryptophan to niacin.

NOTES

Letter to the Editor

Dear Sir:

The thiamine sparing action of some antibiotics (penicillin) is due to their action on the intestinal flora which produce thiamine (*Nutrition Reviews* **17**, *151* (*1959*)). It is not known whether the thiamine synthesized by the intestinal flora is absorbed during "first passage" or during "second passage" after coprophagy. We should like to mention some experiments concerning this question (*Int. Rev. Vitamin Res.* **30**, *376* (*1959*)).

Similar to penicillin, sorbitol was also found to be thiamine sparing, as shown by British, American and German workers. The vitamin sparing action was abolished by addition of sulfaguanidine to the semisynthetic diet containing sorbitol. Furthermore, there was no sparing action during complete prevention of coprophagy, whereas the feeding of feces collected during this time caused a significant increase of body weight.

These results may indicate that extra thiamine produced by stimulation of the intestinal microbiological synthesis by sorbitol is not absorbed during "first passage", but becomes available after coprophagy. This hypothesis corresponds with our experiments on humans, since no thiamine sparing action of sorbitol was demonstrated.

HANS-DIEDRICH CREMER
DIETER HÖTZEL
Giessen, Germany

Vitamin Interrelationships

The July–August number of the *American Journal of Clinical Nutrition* (**7**, *375–443* (*1959*)) is very largely devoted to the papers presented at a Symposium on Vitamin Interrelationships held at the Medical College of Virginia on October 24, 1958.

Of the nine papers presented, four were concerned with folic acid and other pteridines. These covered the following subjects: the role of biopterin (2-amino, 4-hydroxy, 6-dihydroxypropyl-pteridine) in the nutrition and metabolism of the protozoan parasite, *Crithidia fasciculata*, and its relationship to other growth factors, discussed by H. A. Nathan and H. B. Funk; hematopoietic effects of metabolites that depend for their synthesis upon coenzymes containing folic acid, discussed by R. W. Rundles; the production of an uncomplicated deficiency of folic acid in mice by dietary means, presented by G. M. Briggs; and formiminoglutamic acid excretion following a loading dose of histidine, a diagnostic test for folic acid deficiency, presented by A. L. Luhby, J. M. Cooperman and D. N. Teller.

The major emphasis in the remaining papers was on pyridoxine, pantothenic acid and vitamin B_{12} relationships, with a paper on the effects of pyridoxine and pantothenic acid deficiencies on conditioned reflexes by W. H. Gantt, B. F. Chow and M. Simonson, and one on the effect of pyridoxine deficiency on vitamin B_{12} absorption in rats by S. D. J. Yeh and Chow. Vitamin interrelationships in heme synthesis were discussed by D. A. Richert and M. P. Schulman. The role of intrinsic factor in vitamin B_{12} absorption, transport and storage was discussed by V. Herbert.

The remaining paper was a short, speculative but stimulating appraisal by five scientists from the Haskins Laboratories and the Department of Chemistry, Mount Sinai Hospital, New York, of the outlook for studying vitamin interrelationships by microbiological methods.

These papers provide a survey of some of the more recent work on vitamin interrelationships. It is a service to nutritionists to have them published together.

Decreased Density of Bone: An Etiologic Approach to Diagnosis

Recently W. H. Meroney, M. E. Rubini, P. J. Rosch, F. K. Austen, E. G. Herndon, Jr. and W. B. Blythe (*Metabolism* **8**, *293* (*1959*)) have reviewed the metabolism of bone in various disease states (osteoporosis, osteomalacia, and osteitis fibrosa) using the concept of decreased bone density as an etiological approach in diagnosis. The evaluation of bone density was done by viewing the x-rays of the patient's bones.

In this review they discuss the various factors which may influence the calcification of bone, namely, inadequate intake of minerals, inadequate absorption, decreased transportation of nutrients to the bone by the body fluids, diseases of the bone itself (independent of extra skeletal causes), decreased rate of bone formation or increased rate of bone degradation, and renal defects causing increased excretion of calcium, phosphorus or protein. The bibliography of this review has 259 references.

Botulism

Under strictly anaerobic conditions, *Clostridium botulinum* can produce in foodstuffs extremely potent neurotoxins. These toxins constitute one of the most potent poisons known (0.1×10^{-3} micrograms is the LD^{50} for the mouse). Recently Carl Lamanna (*Science* **130**, *763* (*1959*)) has reviewed the present knowledge of the toxins responsible for botulism. Their chemical nature is discussed (proteins consisting only of amino acids), as are their toxic potency, their activity as oral poisons, mechanism of poisoning and the prophylaxis. This review has a good bibliography.

American Board of Nutrition

The American Board of Nutrition will hold the next examinations for certification as a Specialist in Human Nutrition during the week of April 11–15, 1960, in Chicago, Illinois. Candidates who wish to be considered for these examinations should forward applications to the Secretary's office not later than March 1. Application forms may be obtained from the Secretary, Robert E. Shank, Department of Preventive Medicine, Washington University School of Medicine, Euclid and Kingshighway, St. Louis, Missouri.

Recent Books

Principles of Nutrition. Eva D. Wilson, Katherine H. Fisher and Mary E. Fuqua (Pennsylvania State University). Published by John Wiley & Sons, Inc., New York 16, N. Y. Pp. 460. Price $5.95.

Strontium 90 in Human Diet in the United Kingdom, 1958. Report No. 1, Radiobiological Laboratory of the Agricultural Research Council. Her Majesty's Stationery Office, London; British Information Services, New York 20, N. Y. Pp. 75. Price 77¢ postpaid.

Pork in Your Meals. U. S. Department of Agriculture (Agricultural Research Service). Published by the U. S. Government Printing Office, Washington 25, D. C. 1959. Pp. 28.

The Heinz Handbook of Nutrition. Executive Editor, Benjamin T. Burton. Blakiston Division of McGraw-Hill Book Company, Inc. 1959. Pp. 439. Price $5.75.

V.A. Prospectus Research in Aging. Veterans Administration. 1959. For sale by the Superintendent of Documents, Washington 25, D. C. Pp. 125. Price $1.50.

Symposium on Basic Research. Sponsored by the National Academy of Sciences, the American Association for the Advancement of Science and the Alfred P. Sloan Foundation, May 14–16, 1959, New York City. Editor, Dael Wolfle. Published by the American Association for the Advancement of Science, Washington, D. C. Pp. 308. Price $3.00.

BOARD OF TRUSTEES

W. Gardner Barker, *President, Thomas J. Lipton, Inc.*
Claude T. Bissell, *President, University of Toronto*
*F. G. Boudreau, *President, Milbank Memorial Fund.*
William T. Brady, *President, Corn Products Company*
Charles S. Bridges, *President, Libby, McNeill & Libby*
G. Buitoni, *President, Buitoni Foods Corp.*
*Leroy E. Burney, *Surgeon General, U. S. Public Health Service*
*Cabon J. Callaway, *Hamilton, Georgia*
*O. C. Carmichael, *Biltmore, North Carolina*
Thomas P. Carney, *Vice President, Research, Development & Control, Eli Lilly and Company*
Harold W. Comfort, *President, The Borden Company*
George H. Coppers, *President, National Biscuit Company*
W. T. Crighton, *General Manager, Producers Creamery Company*
Wesley M. Dixon, *President, Container Corporation of America*
*Lee A. DuBridge, *President, California Institute of Technology*
*Conrad A. Elvehjem, *President, University of Wisconsin*
Thomas C. Fogarty, *President, Continental Can Co., Inc.*
Edgar J. Forio, *Senior Vice President, The Coca-Cola Company*
*Clarence Francis, *New York New York*
T. R. Gamble, *President, Pet Milk Company*
Daniel F. Gerber, *President, Gerber Products Co.; Vice President, The Nutrition Foundation, Inc.*
George A. Graham, *Secretary-Treasurer, Charles B. Knox Gelatine Co. Inc.*
John A. Grammer, *Executive Vice President, Beech-Nut Life Savers, Inc.*
Henry J. Heinz, II, *Chairman, H. J. Heinz Co.*
*Rev. Theodore M. Hesburgh, C.S.C., *President, University of Notre Dame*
Frank C. Hildebrand, *Vice President, General Mills, Inc.*
*Herman E. Hilleboe, *Commissioner of Health, State of New York*
*Frederick L. Hovde, *President, Purdue University*
Porter M. Jarvis, *President, Swift & Company*
*James R. Killian, Jr., *Chairman, Massachusetts Institute of Technology, Chairman of Board of Trustees, The Nutrition Foundation, Inc.*
*Charles Glen King, *Executive Director, The Nutrition Foundation, Inc.*
*Grayson L. Kirk, *President, Columbia University*
R. Newton Laughlin, *President, Continental Baking Co.*
J. Preston Levis, *Chairman, Owens-Illinois*
R. G. Lucks, *President, California Packing Corp.*
W. F. McLean, *President, Canada Packers, Limited*
William H. McLean, *President of Chemical Division, Merck & Co.*
†Hanford Main, *Chairman and Treasurer, Sunshine Biscuits, Inc.*
Armand May, *Chairman, American Lecithin Co., Inc.*
Oscar Mayer, *Chairman of the Board, Oscar Mayer & Co.*
John T. Menzies, *Chairman, The Crosse & Blackwell Co.*
Charles G. Mortimer, *Chairman, General Foods Corporation; President, The Nutrition Foundation, Inc.*
Milton C. Mumford, *President, Lever Brothers Co.*
W. B. Murphy, *President, Campbell Soup Co.*
H. Earle Muzzy, *Vice Chairman, The Quaker Oats Co.*
William F. Oliver, *President, The American Sugar Refining Company*
*Thomas Parran, *President, Avalon Foundation*
*Maurice Pate, *Executive Director, United Nations Childrens Fund*
Philip W. Pillsbury, *Chairman, The Pillsbury Company*
J. G. Pleasants, *Vice-President in charge of Research and Development, The Procter & Gamble Company*
L. Ratzesberger, Jr., *President, The Illinois Canning Co.*
B. E. Richmond, *President, Richmond-Chase Company*
Lloyd E. Rigler, *President, Adolph's, Ltd.*
Lyle C. Roll, *President, Kellogg Company*
R. T. Ryan, *Executive Vice President, Welch Grape Juice Co., Inc.*
Elbert M. Shelton, *Secretary, The Baker Laboratories, Inc.*
Walter Silbersack, *President, American Home Foods, Inc.*
W. C. Stolk, *President, American Can Company*
Vernon Stouffer, *President, The Stouffer Corporation*
Henry Weigl, *Executive Vice President, Standard Brands, Incorporated*
J. Huber Wetenhall, *President, National Dairy Products Corporation*
F. R. Wilcox, *General Manager, Sunkist Growers*
E. W. Wilson, *Executive Vice President, Armour & Company*
R. D. L. Wirth, *President, Red Star Yeast and Products Co.*
H. J. Wolvlisberg, *President, The Nestle Company, Inc.*
Harry W. Zinsmaster, *Chairman, Zinsmaster Baking Co.*

Horace L. Sipple, *Secretary* John Holmes, *Honorary Trustee*

* Representative of the public
† Deceased.

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.